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Reaction Products from the Chlorination of Seawater

Rosenstiel School of Marine and Atmospheric Science, Miami, FL

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REACTION PRODUCTS FROM THE CHLORINATION OF SEAWATER

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16. ABSTRACT

Current methods underestimate the residual oxidants in chlorinated seawater by as much as 70% depending upon the details of the procedures.

Chlorination of seawater in the presence of light produces bromate ions which can influence standard analytical procedures and represent an unknown factor in estuarine and coastal waters. Toxicity of bromate ion and persistence in coastal waters has not been determined.

The copper complexing capacity of Biscayne Bay, Florida, water was reduced with the addition of chlorine. Analysis was by anodic stripping voltammetry on water samples after successive additions of copper sulfate solutions. Chlorination of seawater may produce toxicity and growth reduction through the indirect mechanism of copper release and/or reduced binding capacity.

Laboratory chlorination of water from the intake of the Port Everglades, Florida, power plant produces bromoform levels comparable to that found in the plant.

Chloroform extracts of chlorinated Biscayne Bay water are found to contain halogenated compounds which are new and different, and which pose unusual analytical problems. Studies using GC/EC, GC/MS, HPLC, ^{1}H NMR, differential pulse polarography and other techniques on natural extracts and synthesized compounds are reported.

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FOREWORD

The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining

- the effects of toxic organic pollutants on individual species and communities of organisms
- the effects of toxic organics on ecosystem processes and components;
- the significance of chemical biocide reaction products in the estuarine and marine environments.

Chemical treatment of natural waters, in particular the use of chlorine as a biocide, modifies the chemistry of these waters in ways that are not fully understood. The research described in this report examined both inorganic and organic reaction products from the chlorination of seawater using a variety of analytical approaches. Conventional methods for the determination of "residual chlorine" were found to underestimate levels in seawater by as much as 70%, depending upon the details of the procedure. It was found that the chlorination of seawater in the presence of light produces substantial quantities of bromate ions which influence standard analytical procedures and represents an unknown toxicity factor in estuarine and coastal waters. Chlorination of Biscayne Bay (Florida) water was found to reduce the copper-complexing capacity which raises the question of whether chlorination may also create toxic effects through the indirect mechanism of copper release or reduced organic binding capacity for ionic copper in addition to direct oxidation and halogen addition reactions. Bromoform was found to be the dominant haloform produced by chlorination of seawater and bromoform was found in the cooling water discharge of the one power plant effluent examined during chlorination. Solvent extracts of chlorinated Biscayne Bay water were found to contain halogenated compounds, primarily brominated, which were resolvable by gas chromatography and detectable by mass spectrometry. Some of these compounds appear to be new and probably are a significant part of "chlorine-produced oxidants" which can be expected to be biologically active. These and other aspects of the determination of reaction products of the chlorination of seawater are discussed in the body of this report.

Henry F. Enos, Director
Environmental Research Laboratory
Gulf Breeze, Florida

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ABSTRACT

A general study of the reaction products from the chlorination of seawater is reported. The results include the following:

Some analytical methods in widespread current use underestimate the residual oxidants in chlorinated seawater by as much as 70% depending upon the detail of the procedures.

The chlorination of seawater in the presence of light produces substantial quantities of bromate ions which can influence standard analytical procedures and represents an unknown factor in estuarine and coastal waters. The toxicity of bromate ion and the possibility of bromate as a persistent source of brominated compounds in coastal waters needs to be determined.

The copper complexing capacity of Biscayne Bay, Florida water was found to be substantially reduced with the addition of chlorine. Analysis was made by anodic stripping voltammetry on water samples after successive additions of copper sulfate solution. The chlorination of seawater may, therefore, produce toxicity and growth reduction through the indirect mechanism of copper release and/or reduced binding capacity.

Laboratory chlorination of water from the intake of the Port Everglades, Florida power plant produces bromoform levels comparable to that found in the plant discharge. These results are in contrast to results reported in the literature for a power plant on the Patuxent estuary in Maryland, so that bromoform production appears to be site-specific.

Chloroform extracts of chlorinated Biscayne Bay water are found to contain halogenated compounds which are new and different, and which pose unusual analytical problems. Studies using GC/ECD, GC/MS, HPLC, ^{1}H NMR, differential pulsed polarography and other techniques on natural extracts and synthesized compounds are reported.

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Support from the Department of Energy also contributed to this work.

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SECTION 1

INTRODUCTION

The toxicant of choice for the control of fouling organisms in power plant coolants and the disinfection of sewage has been chlorine for many years. Most power plants rely upon the continuous or periodic addition of chlorine added as gas or hypochlorite to maintain efficient heat transfer. We estimate that over 100,000 tons of chlorine are consumed annually in a manner that constitutes a probable input to saline water resources. The saline waters that receive these effluents are usually productive near shore estuarine waters that are used extensively by man for recreation and for food.

Chlorine is a desirable pesticide in many respects. Application can be precise, it (or its reaction products) is toxic to many of the organisms that man seeks to control, and it has a limited persistence. It is this very desirable property of short residence time that causes us concern, however. Unlike many pesticides, chlorine is highly reactive in the broadest sense. The variety of possible reaction products is immense and almost entirely unexplored in saline waters, especially for organic reactions. In the research reported herein, we first examined the analytical procedures available for determining "residual oxidants" in seawater, then looked for and found the formation of bromate in sunlight, but not in dark. We then found the most abundant "volatile" product (i.e., removable by gas purge) was bromoform. Subsequently we examined "lipophylic" reaction products using gas chromatography with a variety of detectors including mass spectrometry.

Administratively, this is a "final report," however, in terms of our understanding of the use of chlorine as a pesticide in saline waters, it is an initial contribution to the work that remains to be done as described herein.

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SECTION 2

CONCLUSIONS

. Analytical methods for the determination of "residual chlorine" (more correctly termed "chlorine produced oxidants") developed for freshwater use cannot be applied to saline waters without critical examination. A technique is described that is shown to be applicable to seawater.

. Bromate ion results when seawater is chlorinated in the presence of sunlight. Photochemical reactions, both inorganic and organic, can be expected to play a significant role in chlorination reactions.

. Chlorination of Biscayne Bay, Florida, waters reduces the capability of that water to bind free copper, measured electrochemically. It is probable that the reduction in complexing capacity is due to general oxidation of organic constituents in the water.

. Observations at several coastal plants are needed for a meaningful assessment of the haloform discharge rate that are related to laboratory chlorination studies before prediction of power plant discharges can be made.

. Chloroform-soluble brominated compounds of moderate volatility and molecular weight have been observed in extracts of chlorinated Biscayne Bay water. These compounds, which pose unusual analytical problems, are believed to be an organic fraction of "residual oxidants" that participate in iodometric titration.

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SECTION 3

RECOMMENDATIONS

Determine and observe the uptake of compounds found to result from the chlorination of seawater using selected organisms in EPA controlled-exposure facilities.

Develop techniques for determining selected series of haloamines in seawater at trace levels and examine chlorinated coastal waters for these compounds using HPLC with fluorescence detection at sub-picomolar levels.

Utilize polarography (e.g., differential pulse) to determine electro-reducible compounds in natural saline waters before and after chlorination in conjunction with HPLC/fluorescence measurements.

Study the formation, decomposition and reactivity kinetics of haloamines utilizing sophisticated stop-flow reaction techniques. Equipment for these studies is available at no capital cost to EPA.

Study the reactivity of haloamines with other substrates in living organisms in vitro and vivo. Mutagenesis is of particular concern and can be explored using the Ames test. Coupling reactions with RNA would be particularly interesting and could be sought using C-14 tagged amines.

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SECTION 4

ANALYTICAL PROCEDURES FOR THE DETERMINATION OF RESIDUAL OXIDANTS

With the increasing recognition of the responses of aquatic organisms to low levels of "residual chlorine" (1, 2), measurement of the residual oxidants in the waters discharged from wastewater treatment plants and electricity-generating plants has become important in order that the environmental impact of the discharges can be properly assessed and regulated. Operation of electricity-generating plants frequently involves the use of chlorine as an antifouling agent in the cooling water system, and the large number of plants that have been built at estuarine and coastal sites during the past decade has led to much greater input of chlorinated waters. The wastewater collection systems of many coastal communities contain some seawater as a result of infiltration with brackish ground waters, and chlorine added as a disinfectant during treatment reacts with the seawater constituents during treatment and in receiving water. We find that the analytical methods in widespread current use underestimate the residual oxidants in chlorinated seawater by as much as 70%, depending on the details of the procedures (3).

The addition of chlorine to waters containing sea salts lead to reaction with the natural bromide ion (65 mg/L in ocean water) to produce hypobromous acid and hypobromite ion (4). If ammonia is present, a mixture of monobromamine and monochloramine may be formed (5). In addition, reaction with organic compounds may produce a variety of brominated substances. Thus, the determination of "residual chlorine" actually corresponds to the estimation of the sum of this complex mixture and is better termed "residual oxidant determination."

The toxicity of chlorinated waters has been reported in terms of the combined residual chlorine concentration (1), and the results of iodometric amperometric titration measurements appear to be the most closely correlated with biologically active chlorine residuals (6). Since responses by fish have been found at the low concentrations of 0.001-0.01 mg/L, the high sensitivity of amperometric titration equiment with large electrodes has been attractive. The high sensitivity is attained by vigorous agitation of the sample with the danger that volatile halogen compounds may be lost, so that rapid titration has been recommended. The basic procedure (7) consists of adjusting the pH of the sample to 4 with acetate buffer, adding KI solution (final concentration 0.001-0.003 M depending on sample size), and rapidly titrating the liberated iodine with either sodium thiosulfate or phenylarsine oxide solutions. Similarly, the direct solid electrode amperometry (8) involves the continuous addition of an acetate buffer solution containing KI to the sample stream and the monitoring

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of the current produced by electrochemical reduction of the resulting iodine, with the refinement of using coulometrically generated iodine for periodic calibration. These procedures involving reaction of KI with the residual oxidants during a brief reaction time do not respond to all the residual oxidants in chlorinated seawater.

EXPERIMENTAL

The determination of residual oxidants were carried out using reagent solutions and procedures as described in "Standard Methods" (7), except that the titration of iodine was followed by photometrically using the apparatus and procedure outlined by Carpenter (9). Stock solutions of bromine was prepared by dilution of distilled water saturated with liquid bromine.

The triiodide ion concentration in experimental soltuions was monitored at 350 nm with a Beckman Model 24 spectrophotometer with a recorder.

RESULTS AND DISCUSSION

The results of titrations for residual oxidants in chlorinated seawater showed that Gulf Stream water filtered thorugh 0.22-μ Fluoropore filters had a large apparent chlorine demand, even though such waters have a very low organic carbon content. In addition, the end point of the titrations was not persistent, and triiodide ion was slowly generated after the intial end point has been reached, with 18-24 h required for cessation of additional appearance of triiodide ion. For example, for a chlorine dosage that produced an apparent residual oxidant concentration in distilled water of 4.0×10^{-5} N in two separate trials. The pH 4 buffer and KI results (final solution 0.0024 M KI) were added in less than 1 min after the chlorine dose to minimize reaction with seawater organics or decomposition. The residual oxidants reacted completely with KI in 1 min or less in solutions of pH 2 and 0.024 M KI in distilled water, and the test seawater showed a residual oxidant concentration of 3.6×10^{-5} N under these conditions.

The high "chlorine demand" of the Gulf Stream seawater appears to be due to the rapid formation of chemical species that react slowly with 0.0024 M KI at pH 4.

The addition of chlorine to seawater results in the rapid production of hypobromous acid and hypobromite ion. Based on observation of the 330-nm peak of hypobromite ion, the reaction is complete in less than 1 min. It seemed probable that the slow reacting species was formed from the bromine rather than the chlorine. This possibility was confirmed by use of the simple system of KI solutions in distilled water at various pH values to which an aqueous bromine solution was added and the absorbance to 350 nm (the triiodide peak was monitored with time). The rate of appearance of the triiodide ion depended on the pH of the solutions and KI concentration. For the "Standard Methods" (7) conditions of pH 4 and 0.0024 M KI, 70% of the total potential triiodide ion concentrations appeared in less than 20 s, and further production was very slow. The slow formation of triiodide ion observed photometrically is

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analogous to the fading end point in the iodometric titrations. The inference is that the formation of the slow reacting species is not peculiar to seawater. The initial formation of tiiodide ion was less (30-50%) if the stock KI and buffer solutions were added to brominated distilled water, as in the case of the procedures for analysis of residual oxidants, but was difficult to reproduce with precision. We used the bromination of the diluted KI solution to illustrate the formation and kinetics of the species that reacts slowly with iodide ion.

The rate of production of triiodide ion by the species formed when bromine was added depended strongly on the pH of the solutions (Figure 1). Slow reaction at pH 4 was found even in the presence of a tenfold higher concentration of KI than is normally used in the amperometric procedures for residual chlorine.

We sought to identify the slow reacting species by observing the triiodide ion concentration variation with time after the addition of iodine, chlorine, iodate, and bromate to 0.025 M KI solutions at various pH values (Figure 2). Addition of iodine produced a triiodide ion absorbance nearly instantaneously that did not vary with time or pH, showing that hydrolysis or disproportionation reactions were not responsible for the variations shown in Figure 1. Similarly, the addition of chlorine caused an absorbance that did not vary with time or pH, which supports the notion that the slow appearance of iodine in chlorinated seawater is not caused by reactions involving chlorine.

An obvious species that could cause the observed behavior is bromate ion, since the rate of reaction between iodide and bromate is strongly dependent on acidity (10), and the formation of bromate from bromide by hypochlorite is favored by a high concentration of chloride (11). As shown in Figure 2, bromate reacts with 0.24 M KI only slowly at Ph 2 and does not appear to be the species that causes the results shown in Figure 1. Furthermore, we were unable to detect bromate in chlorinated seawater polarographically, unless the solutions were exposed to sunlight (12).

Addition of iodate to the 0.24 M KI solutions produced patterns of triiodide ion appearance with time at various pH values (Figure 2) that are similar to the results hown in Figure 1, ranging from rapid at pH 2 to very slow at pH 5. We infer that hypobromous acid or bromine (1.4×10^{-5} N) reacts rapidly with iodide (0.024 M) to produce a mixture of iodine and iodate; subsequently, the iodate reacts with the excess iodide to produce additional iodine at rates that depend on the pH.

Confirmation of the formation of iodate from iodide by added bromine at pH 4 was found from differential pulse polarograms. Differential pulse polarography was used in order that the iodate wave could be resolved on top of the large current due to the reduction of the iodine that is also present as a result of the reaction of the iodide with the added bromine.

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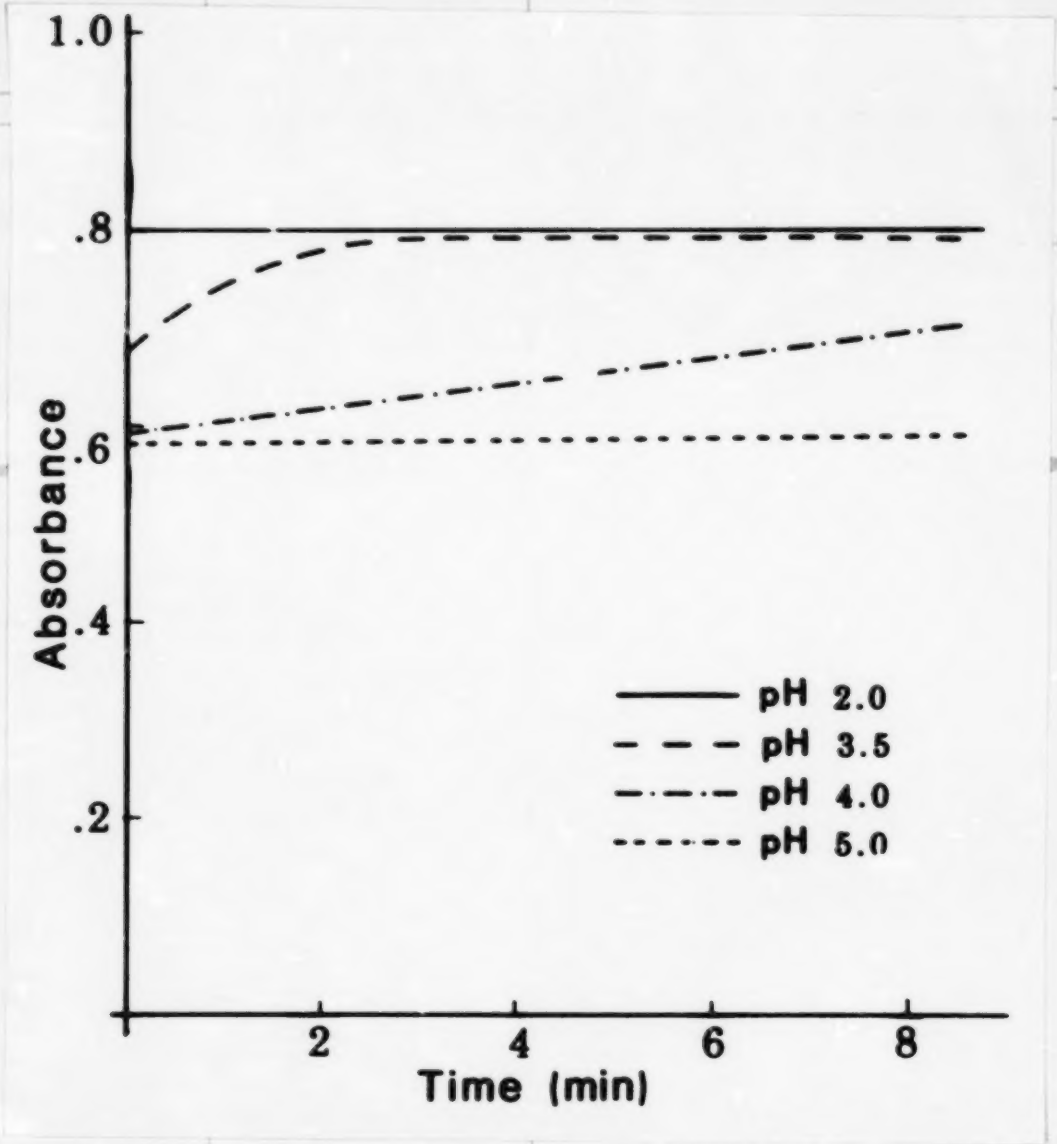

Figure 1. Variation of triiodide ion absorbance (350 nm, 5 cm path) with time after addition of bromine (final solution 14 μN) to 0.024 M KI distilled water solutions adjusted to various pH values with sulfuric acid.

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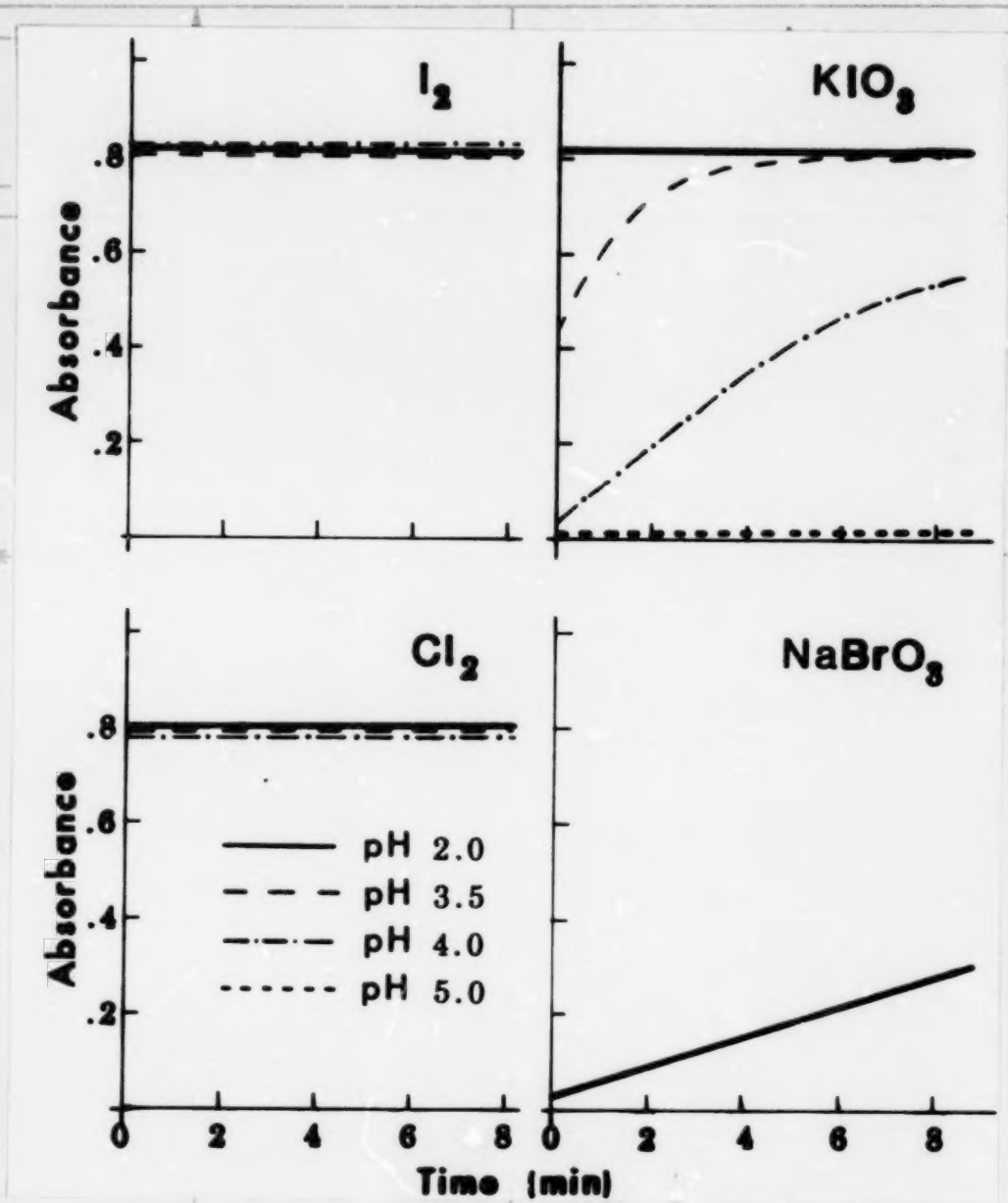

Figure 2. Variation of triiodide ion absorbance (350 nm, 5 cm path) with time after addition of either iodine, potassium iodate, chlorine, or potassium bromate (final solutions 14 μN) to 0.024 M KI distilled water solutions adjusted to various pH values with sulfuric acid. Variation with added iodate similar to variation with added bromine in Figure 1.

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COMPARISON OF ALTERNATE PROCEDURES

These results indicate that the amperometric standard method badly underestimates the residual oxidants in chlorinated seawater because the resulting bromine oxidizes part of the iodide to iodate. In our experiments the residual oxidant was primarily hypobromous acid because Gulf Stream seawater contains very low concentrations of ammonia and organic matter. We considered the ferrous ammonium sulfate titrimetric method with N, N-diethyl-p-phenylenediamine (DPD) end point indicator (13) as a possible aternate procedure for these unpolluted waters. Application of this method to Gulf Stream seawater gave only 80-85% recovery of added chlorine. The reasons for this discrepancy have not been identified. The DPD ferrous titrimetric method does not appear to be suitable for low (0.1-0.01 ppm) concentrations of residual oxidants because the visual end point is not sharp in these dilute solutions.

Another possible procedure would be a modification of the iodometric procedure at lower pH and higher potassium iodide concentrations, so that iodate would react rapidly. Greater acidity and iodide ion concentration should be limited to conditions that do not produce significant air oxidation of the iodide, and pH 2 with ca 0.03 M KI appears to be a realistic limit. The high KI concentration has the additional virtue or reducing the volatilization of the iodine by the formation of the triiodide ion complex, but has the drawback of reducing the amperometric response since the electrode senses primarily the free iodine (14). In the photometric titration procedure that we used, the triiodide ion concetration is measured, and the high KI concentration is desirable.

The results of titrations for residual oxidants in chlorinated seawater, by use of variations of the iodometric procedure, are shown in Table 1. The pH was adjusted and the KI solutions were added within 1 min after the addition of the chlorine solution to minimize the decomposition of the resulting oxidants. Use of pH 4 and low KI concentration produced values that are substantial underestimates, as delineated above. The chlorine disappearance in seawater has been described as involving an initial rapid decline followed by a much slower decline (15), and our results suggest that the apparent rapid initial decline may be partially an artifact of the analytical method.

The use of greater acidity and KI produced values that correspond to a loss of consumption of chlorine in the Gulf Stream seawater of roughly 1 to 2 μeq/L, which is possibly a reasonable result. However, the use of the back titration procedure (6, p. 382), in which the sample was added to an excess of phenylarsine oxide solution that had been mixed with the pH 4 buffer and the KI solution and the excess phenylarsine oxide titrated with a standardized iodine solution, gave results corresponding to 100% recovery of the oxidizing capacity of the added chlorine. The reverse tiration has been recommended for wastewaters to avoid reaction of the liberated iodine with the organic material in such solutions. A possible explanation for the difference between the direct titration results and the back titration results would be the reaction of the iodine with organic matter during the 10 min or so required for the photometric titration that was used. If so, this effect would be greater in

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estuarine and coastal waters that contain much more organic matter than the Gulf Stream seawater.

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The quantitative recovery of the added chlorine oxidizing capacity with the back titration procedure suggests this procedure is suitable for residual oxidant determinations in chlorinated seawater. The procedure has the following advantages:

A low KI concentration may be used without the danger of iodine volatilization, and the response of the amperometric electrodes is not reduced, as it would be if a high KI concentration were required.

The pH 4 acetate buffer is convenient for adjusting the pH of samples.

The high pH and low KI concentration should reduce the possible effects of interference from air oxidation and ferric, manganic, or nitrite ions.

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TABLE 1. APPARENT RESIDUAL OXIDANT CONCENTRATIONS IN DISTILLED WATER AND SEAWATER WITH DIFFERENT ANALYSIS PROCEDURES FOR TWO DIFFERENT CHLORINE ADDITIONS[a]

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Procedure	Residual oxidants, M	Recovery %
a	21.7	...
b	6.7	31
c	18.7	86
d	21.4	99
a	40.6	...
b	21.2	52
c	38.8	95
d	41.3	102

[a](a) Distilled water, direct iodometric at pH 2 in 0.024 M KI; (b) seawater, direct iodometric at pH 4 in 0.0024 M KI; (c) seawater, direct iodometric at pH in 0.024 M KI; and (d) seawater, back titration with standardized iodine solution after excess phenylarsine oxide solution added at pH 4 and 0.0024 M KI.

The large errors that we find with current procedures make it difficult to evaluate and compare various toxicity studies involving chlorine since the actual exposure levels probably have been underestimated substantially. Simple correction for the errors does not appear to be practical because the magnitude of the errors depends on the particulars in the analyses, such as whether pH 4 or 3.5 was used and the rate of titration. A need for careful evaluation of the analytical procedures seems obvious, particularly for water containing higher concentrations of ammonia and organic compounds than Gulf

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SECTION 5

SUNLIGHT-INDUCED BROMATE FORMATION IN CHLORINATED SEAWATER

Chlorine and its compounds have been used for water disinfection and as general aqueous biocides in increasing quantities since the turn of the century. The popularity of these materials stems partly from the remarkable apparent tolerance of mammals to them (16) at concentrations that produce mortality of organisms ranging from bacteria to fish; that is, it kills them, not us. Recent estimates (17) indicate that more than 100,000 tons of chlorine are used annually for the partial disinfection of effluents from wastewater treatment plants, and such may be expected to increase substantially as the secondary treatment systems mandated by Congress in Public Law 92-500 begin operation. An additional major use of these compounds is as antifouling agents in the cooling waters of electric generating plants. Somewhat more chlorine is used for this purpose than for wastewater treatment, based on a cooling water flow of 300,000 cubic feet per second (8400 m^3/sec) (18) and a dose of 0.5 mg of Cl_2 per liter.

The release of chlorinated waters is producing effects that are slowly being better documented as a result of continuing research. Summaries of current knowledge (19) show avoidance behavior and reproductive failure in many freshwater invertebrates and fish at chlorine concentrations of 0.003 to 0.005 mg/liter. Federal and state regulations have been used on measurements of "residual chlorine" for both control of wastewater treatment (in the state of Virginia, chlorine is added until the concentration is effluent is 0.2 mg/liter) and effluent limitations on power plants. Considering the strong sensitivity of aquatic organisms to "residual chlorine" and the present levels of chlorine use, substantial damage to aquatic resources may occur. For example, the present releases of chlorine to Chesapeake Bay and its tributaries would sterilize the whole system if there were not environmental degradation of the added chlorine. However, transformation of chlorine to persistent, but less acutely toxic, compounds may be hypothesized to produce slow changes in the abundance and diversity of aquatic species in such situations.

Degradation is operationally defined as the disappearance of the analytical signal for "residual chlorine." As pointed out by Eppley et al. (15), different analytical methods produce very different estimates of "residual chlorine." In fact, the products from chlorination of wastewaters and natural waters are a mixture of chlorine, hypochlorous acid, hypochlorite ion, inorganic and organic chloroamines, and other compounds. A better term is "residual oxidants," as noted elsewhere in this report.

Since a large fraction of the U.S. population resides in coastal areas,

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much of the chlorine is discharged to saline natural waters. There is an extensive literature (19) on chlorination of freshwater systems, but coastal and estuarine waters have not been studied extensively. Research programs are underway at several federal and university marine laboratories to alleviate this situation. The work on freshwaters, unfortunately, does not have much application to marine environments, because seawater has a bromide ion concentration of 65 mg/liter and the added chlorine reacts with it to produce hypobromous acid and hypobromite ion. Bromoamines and chloroamines may be formed in the presence of ammonium ion (20).

For normal seawater of pH 8, the initial products of chlorinations are a mixture of hypobromous acid and hypobromite ion. Both of these compounds are unstable with respect to decomposition and disproportionation (23).

$$2HOBr \rightarrow 2H^+ + 2Br^- + O_2; \qquad (1)$$

$$2OBr^- \rightarrow 2Br^- + O_2$$

$$3HOBR \rightarrow 3H^+ + 2Br^- + BrO_3^-; \qquad (2)$$

$$3OBr^- \rightarrow 2Br^- + BrO_3^-$$

The rates of these reactions have not been measured in seawater. The decomposition of $HOBr-OBr^-$ solutions has been considered most recently by Lewin and Avrahami (21) and by Engel et al. (22). Both groups conclude that the decomposition is to bromate plus bromide, with the disappearance of $HOBr-OBr^-$ or bromide ion concentration and to decrease strongly with increasing pH (22). It is observed to be independent of the chloride ion concentration up to 0.5M (21). No photolytic effects were investigated.

Previous investigators (15, 19) considered only the rate of disappearance of residual oxidants in chlorinated seawater and did not identify the products. The initial rapid decline was ascribed to reactions with organic compounds and the ensuring slower decline to "decomposition." No attention was given to photolysis by laboratory lighting or, more important, by natural sunlight. We report here our observations of residual oxidant disappearance and bromate formation, with particular reference to the significance of photolysis.

Chlorinated seawater was exposing to sunlight in open beakers placed in a bath of running seawater. In each experiment, six 400 ml beakers, each containing 300 ml of filtered (Millepore, 0.22 μm) Florida Current water, were placed in a batch. After temperature equilibrium was reached sufficient NaOCl solution (buffered to pH 8.1 with Na_2CO_3) was added to each beaker to give an initial OCl^- normality equivalent to approximately 4.5 mg Cl_2 per liter. Actual initial OCl^- concentrations varied somewhat (4.2 to 4.9 mg of Cl_2 per liter) among experiments because of varying OCl^- concentrations in the stock solution. Florida Current water contains less than 1 μm NH_3, so formation of haloamines cannot take up more than 5 percent of the added Cl_2.

Light intensities were estimated with a Yellow Springs Instrument - Kettering Model 65A radiometer, operated with the focusing head removed from

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the sensing element. This makes it possible to measure relative light intensities from day to day without the extreme angular dependence caused by the focusing head. Experiments were conducted under full sun, partial sun, and heavily overcast conditions.

After chlorination, beakers were removed from the sunlight at regular (usually 30 minute) intervals, placed in a dark box, and analyzed for bromate and residual oxidants without delay. Residual oxidants analyses were performed by the I_3^- spectrophotometric titration procedure described by Carpenter (9) with a pH of 2 and a KI concentration of 4 g/liter. Bromate analyses were made by differential pulse polarography at 25°C and a pH of 8.35 (after O_2 stripping with N_2), using a Princeton Applied Research Model 174A polarographic analyzer.

A typical polarographic recording is shown in Fig. 3. Curve "a" is the polarogram obtained for chlorinated seawater analyzed immediately after chlorination. Identical traces were observed for nonchlorinated seawater and for chlorinated seawater kept in the dark for periods up to 24 hours at temperatures up to 40°C> which indicates a lack of bromate formation under these conditions ($BrO_3^- \leq 10^{-7}$ M, less than 0.5 percent conversion of Cl_2). Addition of copper sulfate to give a Cu^{2+} concentation in the seawater of 100 parts per billion did not induce measurable bromate production in the dark. Curve "b" was obtained from a chlorinated (4.9 parts per million (ppm)) seawater solution that was exposed to full sunlight for 70 minutes. Curve "c" which is offset by 0.4 μa with respect to curves "a" and "b." shows 1.0×10^{-5} M sodium bromate in seawater.

Figure 4 illustrates kinetic data for the appearance of bromate (Fig. 4A) and disappearance of residual oxidants (Fig. 4B) in chlorinated seawater exposed to sunlight. Curves "a" were obtained from solutions exposed to full midday sunlight for the duration of the experiment; curves "b" are for exposure to partial sunlight (the average light intensity was approximately 65 percent of full sunlight); and curves "c" are for overcast conditions (average light intensity, 20 percent of full sunlight). Curve "d" in Figure 4A shows the disappearance of residual oxidants with time at 40°C in the dark. The ordinates are calibrated as the percentage of the added chlorine recovered as residual oxidants (Fig. 4A) or as bromate formed according to Equation 2 (Fig. 4B).

The lack of observable bromate production in the dark is not inconsistent with the report of Lewin and Avrahami (21) that substantial bromate was formed in their 0.05M hypobromite solutions. Our solutions, which correspond to chlorine use, were 1000 times more dilute. Using their rate constants, we calculate in our solutions a conversion to bromate of less than 1 percent after 24 hours.

The loss of residual oxidants does not correspond exclusively to bromate formation, and other reactions including oxidation of organic matter and perhaps those in Equation 1, also take place. The rate and extent of bromate formation depend on the intensity of sunlight.

In another experiment, 1.0×10^{-5} M solutions of sodium bromate in seawater were exposed to full midday sunlight for periods up to 4 hours and the residual

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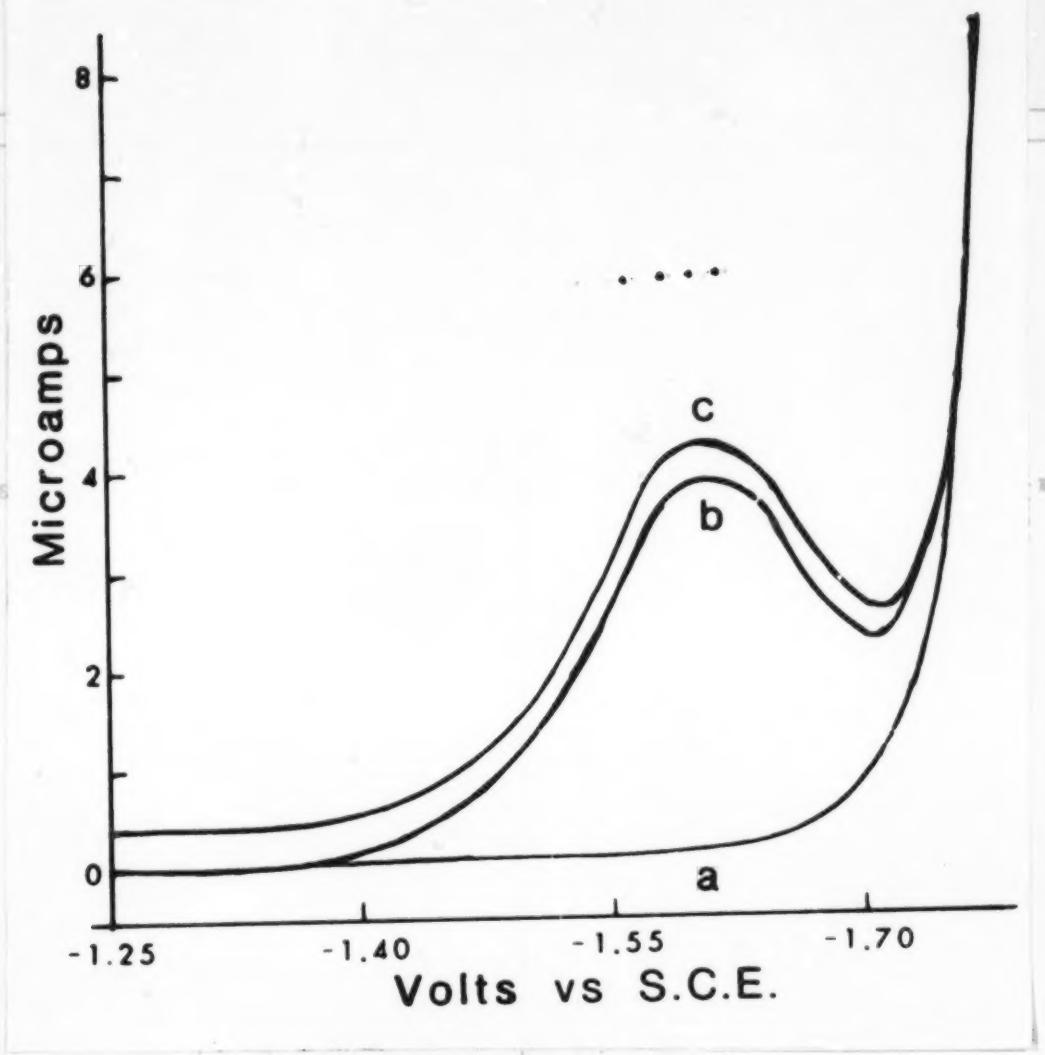

Figure 3. Differential pulse polarographic verification of sunlight-induced bromate production in chlorinated seawater. (Curve a) Polarogram from untreated seawater, seawater immediately after chlorination to 4.9 ppm, or chlorinated seawater kept in the dark for 4 hours at 40°C. (Curve b) Polarogram from chlorinated seawater exposed to full sunlight for 70 minutes. (Curve c) Standard: 1.0×10^{-5}M sodium bromate in seawater, offset with respect to curves a and b. Polarogram were recorded at 25°C and pH 8.35; SCE, saturated calomel electrode.

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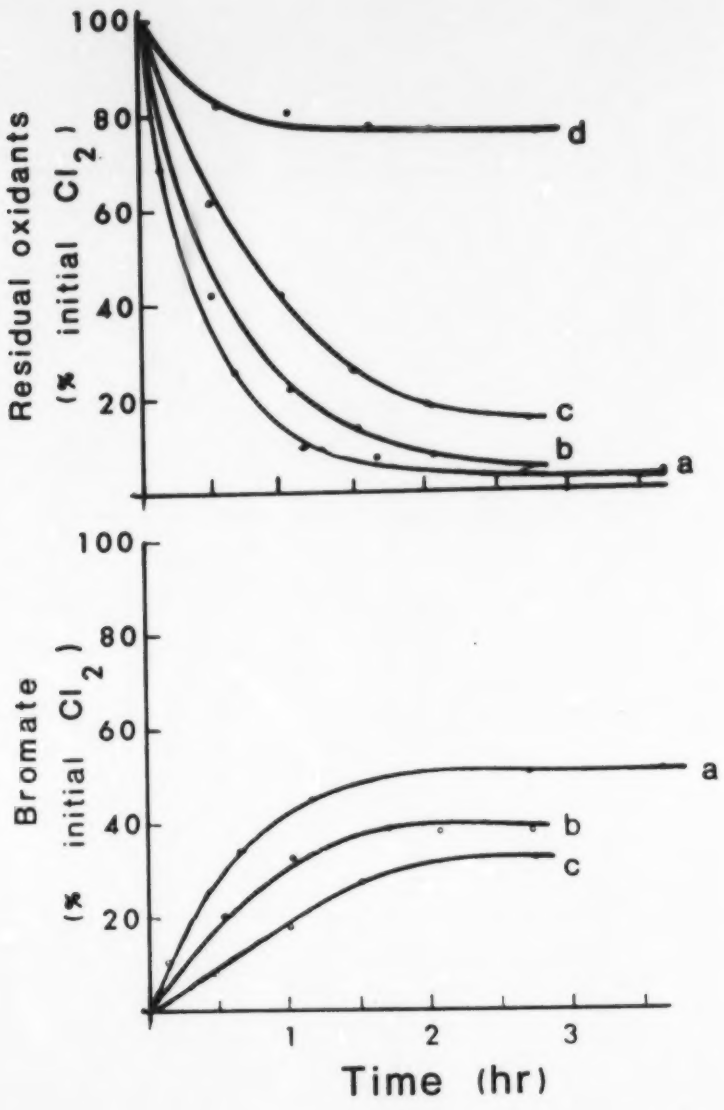

Figure 4. (A) Disappearance with time of residual oxidants and (B) concomitant appearance of bromate (Eq. 2) in chlorinated seawater (4.2 to 4.9 ppm of Cl_2) as a function of exposure to sunlight. The conditions were: (curve a) full midday sunlight, (curve b) 65 percent of full sunlight, and (curve c) overcast, 20 percent of full sunlight. Curve d shows residual oxidant disappearance in the dark at 40°C. No bromate production was observed in the dark.

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oxidant and bromate concentrations were monitored. No measurable decline in bromate concentration or increase in residual oxidant was found.

Thus, the production of substantial amounts of bromate ion will cause erroneous results when standard analytical procedures are used for residual oxidants, especially procedures involving reaction of the oxidants with iodide ion. Bromate reacts sluggishly with iodide ion and the rate is dependent on factors such as reactant concentrations, pH, temperature, light, and content of transition metals. More important, it appears that large amounts of bromate have already been produced in estuarine and coastal waters with unknown effects. Extremely limited information is available on the direct toxicity of bromate ion (19). Further, the formation of bromate may provide a persistent source of low levels of known toxicants (such as hypobromite and bromoamines) and brominated organics through the reverse of the formation reactions. In summary, present knowledge is totally inadequate to assess the environmental impacts of our discharge of chlorine to saline waters.

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SECTION 6

CHLORINATION AND THE COPPER COMPLEXING CAPACITY OF SEAWATER (24)

While copper has long been recognized as an essential micronutrient for the growth of aquatic plants, recent research has drawn attention to the extreme sensitivity of microalgae to free ion in seawater (25) and freshwater (26). Reduction in growth was reported at the nanomolar level for several species. Addition of chlorine has the potential for modifying the organic compounds that nearly completely complex copper in natural waters and thus increase the toxicity of the copper presence. The results of some initial experiments to explore this possibility are reported here.

PROCEDURE

Anodic stripping voltammetry (27) provides a technique for measuring copper with a minimum of sample manipulation or addition of reagents. Biscayne Bay water samples were placed in an electrolysis cell and dissolved oxygen was removed by bubbling argon through the sample for 20 min. The electroactive copper was plated out on a hanging mercury drop electrode (PAR Model 9323) during 10 min with an applied potential of minus 0.8 V vs SCE, using a Princeton Applied Research Model 174 Polarographic Analyzer (Princeton Applied Research Corp., Princeton, N.J.). Then the potential was made anodic at the rate of 2 mV/sec and the resulting stripping current peak due to copper at minus 0.185 V was recorded. Aliquots of a standard copper sulfate solution were added to the electrolysis cell and the copper stripping current measured after each addition to produce a titration of the copper complexing capacity of the sample.

RESULTS

Figure 5 is a plot of the copper stripping currents in a sample of Biscayne Bay water with copper added in increments corresponding to 1 ppb in the sample solution. The initial increments of added copper produced some increase in the copper stripping current and, then, additional increments produced a more rapid increase in the copper stripping current. The initial increase in the copper stripping current may be ascribed to electroactive (labile) copper complexes. The sharpness of the transition between the two slopes suggests that a complex with a very large formation constant is present.

Figure 6 shows the titration of a sample of the same water after chlorination (1.5 ppm Cl_2 added). The copper complexing capacity is reduced.

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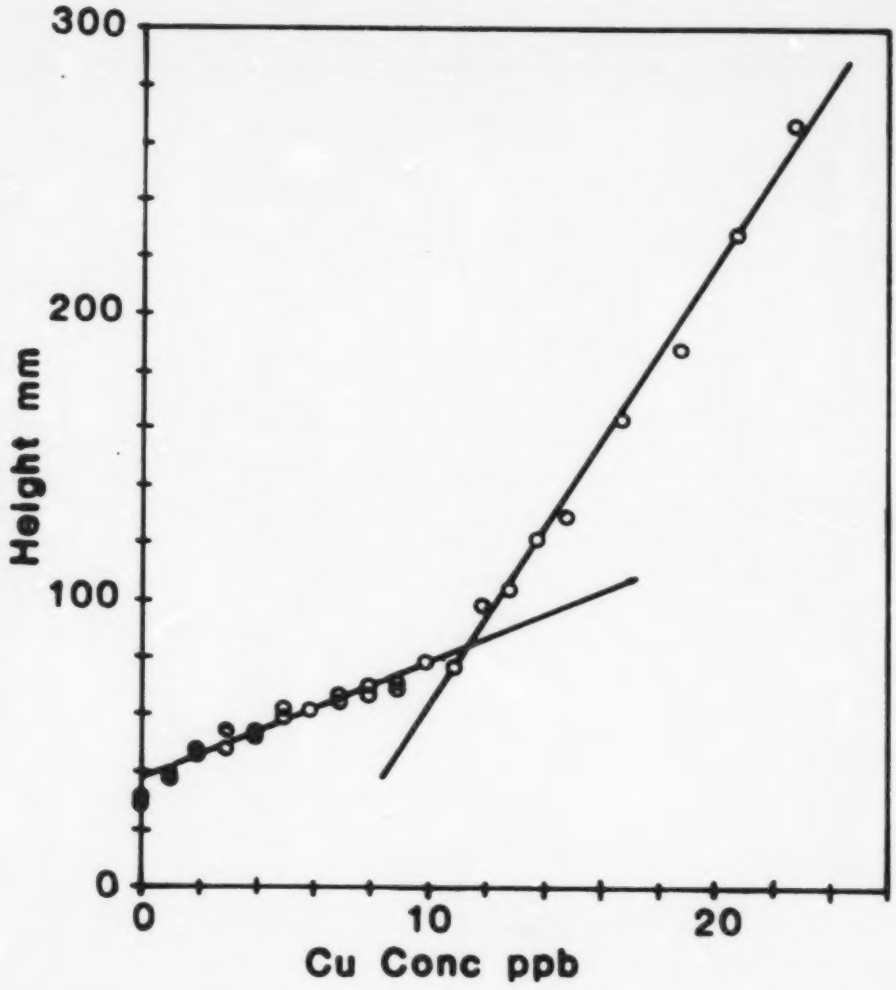

Figure 5. Anodic stripping current variation with copper added to a sample of Biscayne Bay water, showing copper complexing capacity of 12 ppb.

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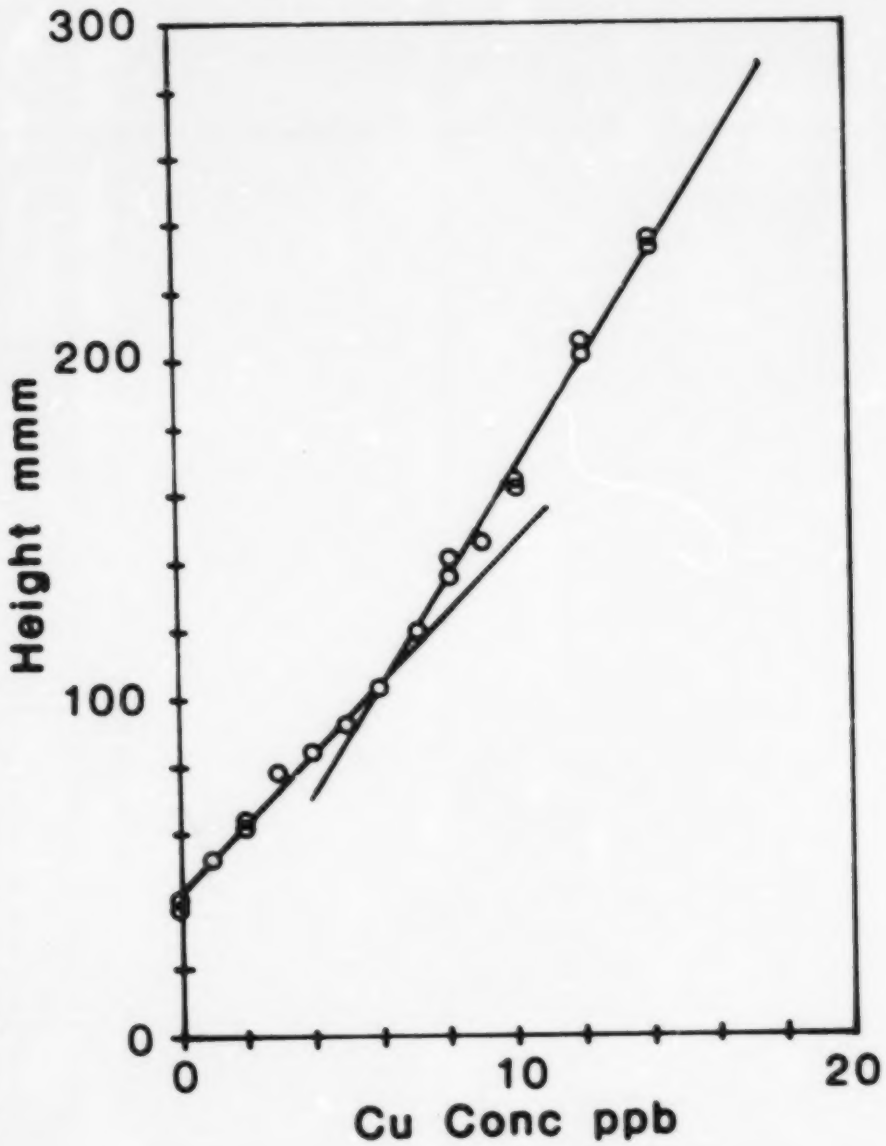

Figure 6. Anodic stripping current variation with copper added to a chlorinated (1.5 ppm Cl_2) sample of Biscayne Bay water, showing decreased copper complexing capacity and changed character of the complexes (same water as in Figure 5).

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Also, the copper complex that remains is altered, as shown by the increased slope of the curve before the endpoint.

Figure 7 shows the titration of another sample of Biscayne Bay water which had a copper complexing capacity of 6 ppb. An aliquot of this sample was chlorinated (5 ppm Cl_2 added). This dosage did not produce a measurable, persistent residual oxidant concentration. This sample was titrated with results that are plotted in Figure 8. The addition of chlorine removed all the measurable copper complexing capacity from this water.

These results suggest that chlorination of seawater may produce toxicity and growth reduction through the indirect mechanism of modifying the copper complexing capacity of the treated water.

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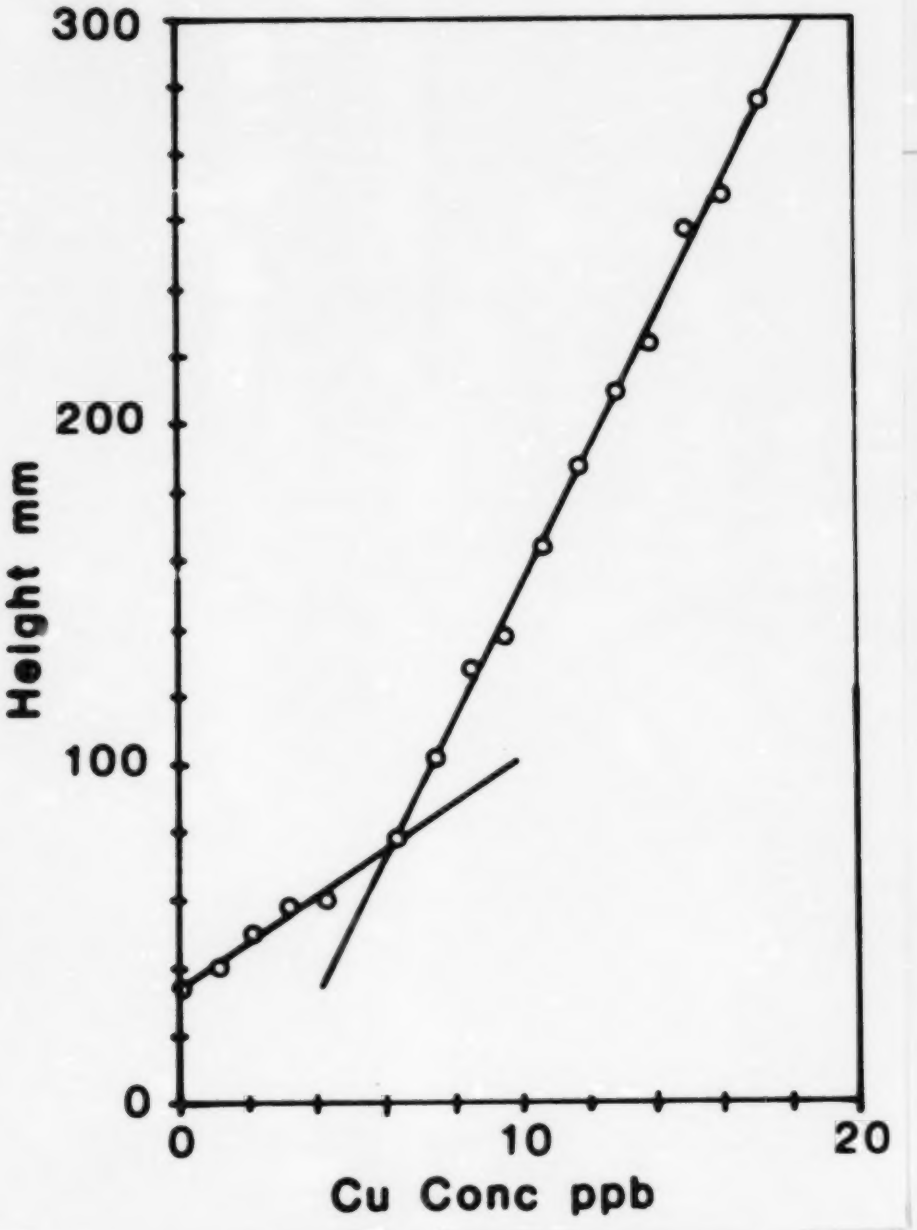

Figure 7. Anodic stripping current variation with copper added to a sample of Biscayne Bay water, showing copper complexing capacity of 6 ppb.

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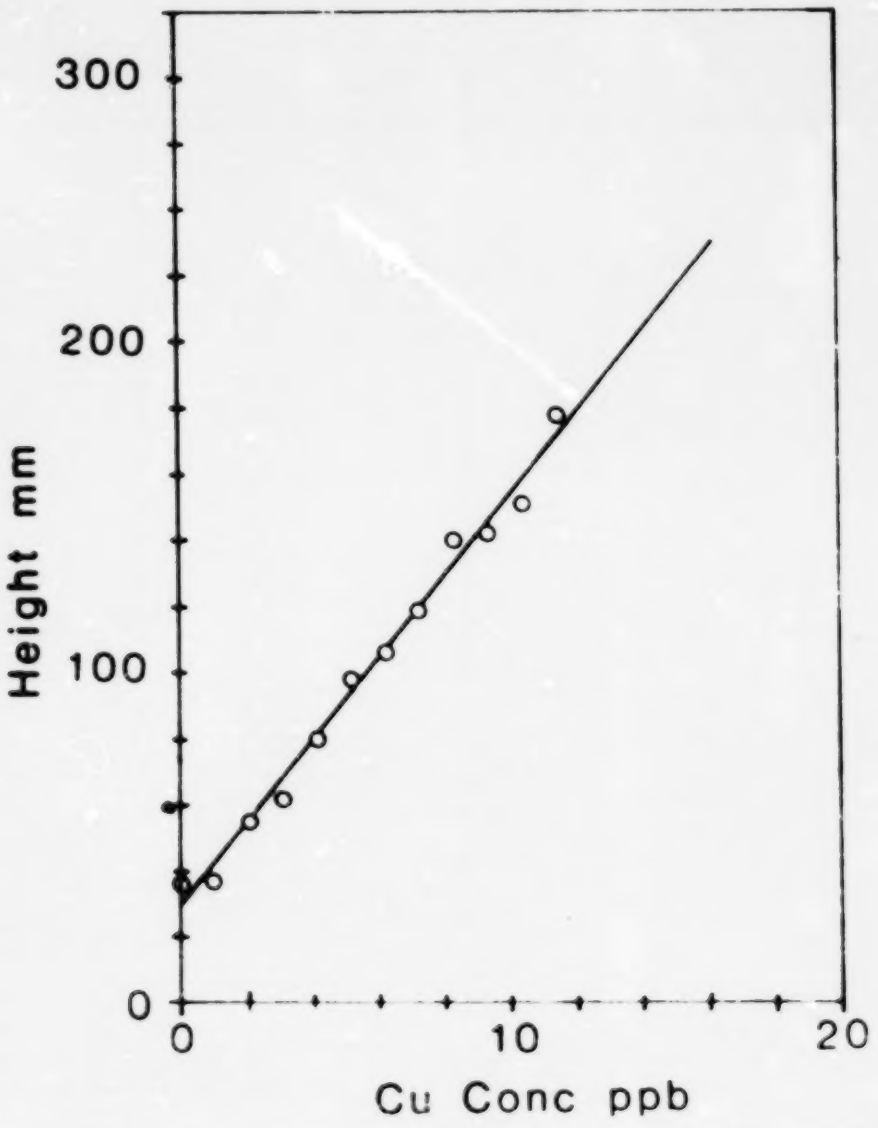

Figure 8. Anodic stripping current variation with copper added to a chlorinated (5 ppm Cl_2) sample of Biscayne Bay water, showing a loss of complexing capacity.

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SECTION 7

A POWER PLANT STUDY: CHLORINATION AT THE PORT EVERGLADES, FLORIDA, POWER PLANT (28)

Much of the present information on the products formed when seawater is chlorinated is based on observations of laboratory experiments in which chlorine was added to seawater to simulate conditions of electricity generating plants. The work of Helz et al. (29) is the only comparison of laboratory simulation and direct sampling at an operating power plant that we have found in the literature. They found that chlorine-produced oxidants disappeared to a much greater extent (factor of ten) in the water that passed through the Chalk Point plant than did an equal oxidant dose under laboratory conditions or in samples withdrawn from the plumbing upstream of the condensers inside the power plant. Also, they measured only traces of haloforms in the discharge canal waters, even though 10-100 µg/l $CHBr_3$ was formed in chlorinated intake water. On some of their sampling dates, ammonia nitrogen was found to be higher in the discharge canal than in the surface water at the intake, but this feature was not observed under laboratory conditions. These discrepancies led Helz et al. to the view that "laboratory dosing of water from the intake canal with 1-2 mg/l Cl as NaOCl provided a poor model for what was observed in the field."

Sampling at the Port Everglades power plant of the Florida Power and Light Company was undertaken for comparison with the observations at the Chalk Point plant on the Patuxent estuary in Maryland by Helz et al. The Port Everglades power plant consists of four oil-fired units with four condensers per unit. Cooling water is pumped from the harbor and during observation had salinities of 29.5-31.1 parts per thousand (ppt), in contrast to the average salinity at the Chalk Point plant of 5 ppt. Microorganism fouling of the condensers is not a substantial problem at Port Everglades, and chlorination for 15 min/day on each condenser sequentially at a dose of 0.6 ppm is used. Samples were collected from the pipe just downstream of the circulating water pump (chlorine is injected through a manifold in the intake structure and mixing takes place in the pump) and from the discharge sluiceway. Mixing of chlorinated and unchlorinated water takes place in the discharge sluiceway and the resulting nonuniformity produces variability in replicate samples. Additional samples at the circulating pump were held in glass flasks for 2.5 min and then stabilized for comparison with the water collected at the discharge that passes through the plant in approximately 2.5 min.

METHODS

The samples for residual oxidant determinations were stabilized

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immediately after collection by adding phenylarsine oxide (PAO) solution, and the unreacted PAO was titrated with standard iodine solution using photometric endpoint detection (3) within 24 hr of collection. Bromoform concentrations were determined using a purge-and-trap procedure (30) with a Hewlett-Packard 5730A chromatograph and electron-capture detection (ECD). The samples for the ammonia nitrogen determinations were stabilized at collection by adding phenol solution and analyzed using the Solorzano procedure (31).

RESULTS

Comparison of the concentrations of residual oxidants in the discharge water and in water from the circulating water intake pump held for 2.5 min did not show a remarkable difference in the rate of disappearance of the residual oxidants. The decrease in residual oxidant concentration in either case was typically 50%. As shown in Table 2, bromoform remained essentially constant within a factor of two during travel through the power plant. (Intake water was held for 2.5 min before quenching to allow for passage time through the plant.) Due to physical factors in the plant, there was both some periodic and uncontrolled mixing at the intake and discharge of the plant, but these analyses were made on water taken as close to intake and discharge as possible. Laboratory chlorination of water taken at the plant site produced varying levels of bromoform, but at 4 ppm added oxidant, we did not find the 10X excess found by Helz as shown in Table 3. (We do not have a measure of chlorine added at the plant.)

TABLE 2. BROMOFORM IN PORT EVERGLADES, FLORIDA, POWER PLANT INTAKE AND DISCHARGE WATERS

Date	Unchlorinated Intake Water	Chlorinated* Intake	Chlorinated* Discharge
12 Sep 1980	1 ppb	75 ppb	86 ppb
20 Oct 1980	1 ppb	78 ppb	32 ppb

* Chlorine residual was 0.5-0.6 ppb.

Ammonia nitrogen in the intake water samples average 25 µg/l. Similar values were observed in the discharged waters and in the intake samples that were held for 2.5 min before addition of the phenol solution.

Observations show no major effects associated with passage of the cooling water through the Port Everglades plant. Comparison with the results of Helz *et al.* for the Chalk Point plant suggests there must be some site-specific considerations that have not been identified. The total organic carbon (TOC) at both sites was approximately 5 mg/l and the ammonia nitrogen levels were comparable; thus, gross water quality was not strikingly different. The condensers at Chalk Point have copper-nickel tubing and those at Port Everglades have aluminum-brass tubing, but there is no information on the

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reactivity of chlorinated water with these materials. Observations at several coastal plants are needed for assessment of the haloform discharge rates and usefulness of laboratory chlorinated studies for anticipating the nature of power plant discharges.

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TABLE 3. LABORATORY CHLORINATION OF POWER EVERGLADES POWER PLANT INTAKE AND DISCHARGE WATERS

Chlorine Added	$CHBr_3$ Detected
1 ppm	6.5 ppb
2 ppm	107 ppb
4 ppm	272 ppb

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SECTION 8

LIPOPHYLIC HALOGENATED COMPOUNDS: THE LIQUID/LIQUID EXTRACTABLES

8.a. INTRODUCTION

Bromoform was found to be the major "volatile" halogenated compound produced by the chlorination of seawater, measured using the purge-and-trap method. However, bromoform (and its one and two carbon halogenated analogues) cannot account for more than a small percentage of the total organic carbon consumed. It was postulated that halogenated reaction products which would not extract by sparging with inert gas but would partition favorably into solvents such as ethyl ether or chloroform (i.e., lipophylic in nature) were present in the chlorinated saline waters studied. Lipophylic halogenated compounds are of particular interest due to their capacity for incorporation into fatty tissue and their transport across cell membranes. Fish, for example, would be expected to assimilate such compounds through the gut and across gill membranes with possible accumulation in fatty tissue being transferred up the food chain. Such compounds could have long-term environmental effects while not necessarily demonstrating large LD/50 values for mature or perhaps even larval stage organisms, in a food chain sequence. Such compounds would be expected to partition favorably into halogenated hydrocarbons such as chloroform. In addition, we would expect that some of these compounds would behave as mild oxidants making them susceptible to reducing agents such as potassium thiosulfate. We chose, therefore, to look for such compounds using large volume extracts of chlorinated Biscayne Bay water, first using chloroform and later using ethyl ether as the extraction solvents. Initially, the study of lipophylic halogenated compounds centered on GC/MS analysis of chloroform extracts of chlorinated Biscayne Bay water. Mass spectral analysis showed that the compounds observed were not similar to compounds in mass spectral libraries, but definitely were brominated and possibly contained nitrogen. One interpretation suggested a compound such as bromopyrrolidine although such a compound is not recorded in the literature. Subsequently, attempts to synthesize a compound with the same mass spectrum were performed using proline which was considered to be a potential precursor. This resulted in intermittent low yields of compounds that matched the mass spectra and retention times of those found in natural extracts. Suspecting cyclic amino acids to be possible precursors, a variety of amino acids were chlorinated in a seawater matrix and chromatographed using HPLC with fluorometric detection. The unknown compounds proved to be stable in solvent solutions but virtually impossible to purify by the usual methods such as adsorption chromatography, distillation, etc. Milligram quantities of the compounds were obtained by a small capacity preparative gas chromatograph and this set up was used to generate enough sample for proton NMR and elemental analysis.

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8.b. HEXANE AND CHLOROFORM EXTRACTIONS

ECD-Capillary Column Results

Early in the study of liquid/liquid extractables, a comparison was made between hexane(s) soluble and chloroform soluble fractions. As shown in Figure 9, chloroform extracts of Biscayne Bay water, both chlorinated and unchlorinated, contained a much larger number and quantity of compounds detectable by the electron capture detector (ECD) than did hexane extracts obtained in a similar manner. In this case, the experiment consisted of the collection of 4 carboys of 16 liters each of Biscayne Bay water, two of which were chlorinated to ca 5 ppm. Extraction was done in tandem, i.e. the raffinate from the hexane extraction was fed directly into the chloroform extractor and the extracts treated equally. The process took several days to complete so the chlorine (as chlorine saturated water) was added 30 min from the time the extraction was started. The water was not filtered but was acidified to pH 5 just before extraction to reduce foaming. The hexane fraction contained few compounds detectable by the ECD and a much more sensitive gain level (X128) was required, compared with the chloroform extract (X2048). GC/MS on the hexane extract produced no usable spectra as would be expected from lower sensitivity of that instrument (in the broad scan mode) compared with the ECD. GC/MS on the chloroform extract did, however, show compounds that are of great interest.

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GC/MS Results

The first chloroform extracts of chlorinated Biscayne Bay water were made on 14 December 1977 and on 30 January 1978; both contained compounds having unusual mass spectra. The 14 December 1977 experiment was similar in execution to the 30 January 1978 run described below except that only $CHCl_3$ was used as solvent and that more attention was given to the compounds more volatile than bromoform. As shown in Table 4, eights peaks were observed to have usable mass spectra from the GC/MS of whch five were tentatively identifiable by comparison with published mass spectra. The halogenated methane and ethane compounds were expected based on previous volatiles analyses and not considered particularly noteworthy. The compounds that eluted at 4.1-4.2 and 5.8-5.9 min had mass spectra that did not match known published spectra and were major in abundance. These two compounds, and in particular the one that eluted at 5.8-5.9 min, were studied extensively.

The total ion chromatogram of the $CHCl_3$ extract of chlorinated 30 January 1978 Biscayne Bay water (Figure 10) contains 18 discernable peaks, of which 8 are too small to give usable mass spectra. Bromoform, at 3.7 min, overlaps with another smaller peak at 3.8 min and a contaminant, probably methylisobutyl ketone is recorded at 2.7 min. The remaining 7 peaks are all halogenated compounds, as shown by the characteristic M, M+2 couplets in the mass spectra shown in Figures 11-13. Even though the mass spectrometer was carefully calibrated by using perfluorotributylamine (PFTBA) and decafluorotriphenylphosphine (DFTPP) in the manner described by Eichelberger et al. (32), none of these spectra are recorded in the Aldermaston Eight-Peak Index (33) or the Cornell-McLafferty mass spectral libraries.

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TABLE 4. TABULATED GC/MS CHROMATOGRAMS OF CHLOROFORM EXTRACTS OF CHLORINATED BISCAYNE BAY WATER

Retention Time, min	Compound	Spectrum, Mass (abundance)
	Run 5076	Chlorinated Baywater, 14 December 1977
1.7	CCl_4	119(100), 117(99), 121(31), 47(22)
1.8	$CHCl_2Br$	83(100), 85(66), 47(34), 48(19)
2.3		45(100), 69(99), 91(79), 53(32), 148(10), 150(10)
2.4	$CHClBr_2$	129(100), 127(83), 131(25), 81(19)
2.7	$C_2H_2Cl_4$	166(100), 164(82), 168(49), 129(42), 131(41)
3.7	$CHBr_3$	173(100), 171(55), 175(51)
4.1		105(100), 69(69), 107(37), 53(20), 149(20), 151(20)
5.8		151(100), 149(99), 69(54), 53(11)
	Run 5078	Chlorinated Baywater, 30 January 1978
2.9	$C_2H_2Cl_4$	166(100), 164(69), 168(46), 129(38), 131(35)
3.7	$CHBr_3$	173(100), 171(55), 175(51)
3.8		43(100), 59(95), 153(37), 151(37)
4.2		105(100), 69(65), 107(36), 53(19), 149(15), 151(12)
5.9		149(100), 151(99), 69(44), 53(10)
9.6		43(100), 99(57), 55(28), 42(9), 135(8), 137(7)
10.0		137(100), 139(95), 43(48), 151(39), 153(36)
10.5		139(100), 137(96), 153(73), 151(72), 43(72)
11.9		185(100), 183(75), 67(40), 103(35), 147(29), 149(31) 227(8), 229(10), 231(8)
12.2		185(100), 182(81), 67(42), 103(38), 147(32), 149(38)

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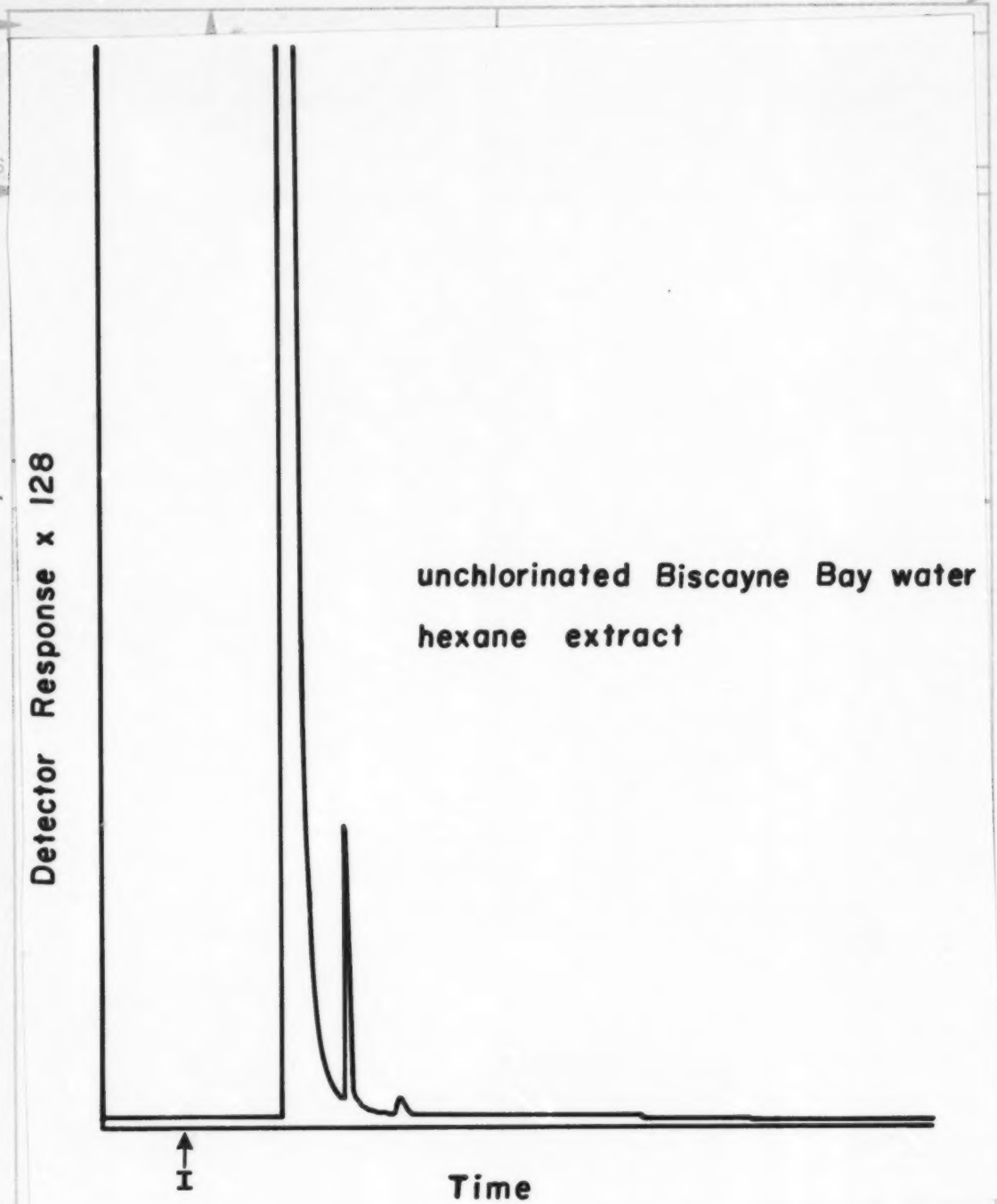

Figure 9a. GLC chromatogram of hexane extract of unchlorinated Biscayne Bay water.

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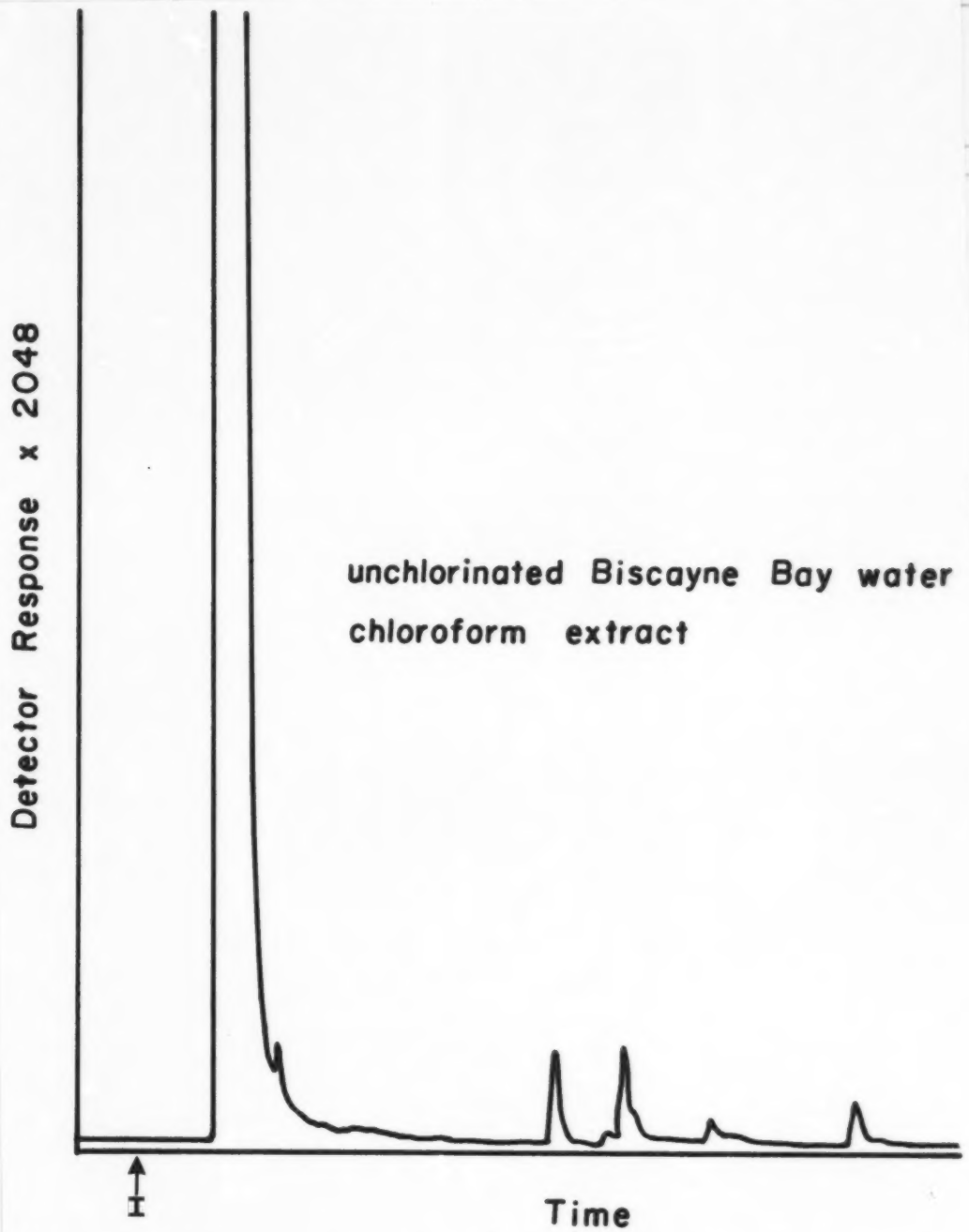

Figure 9b. GLC chromatogram of chloroform extract of unchlorinated Biscayne Bay water.

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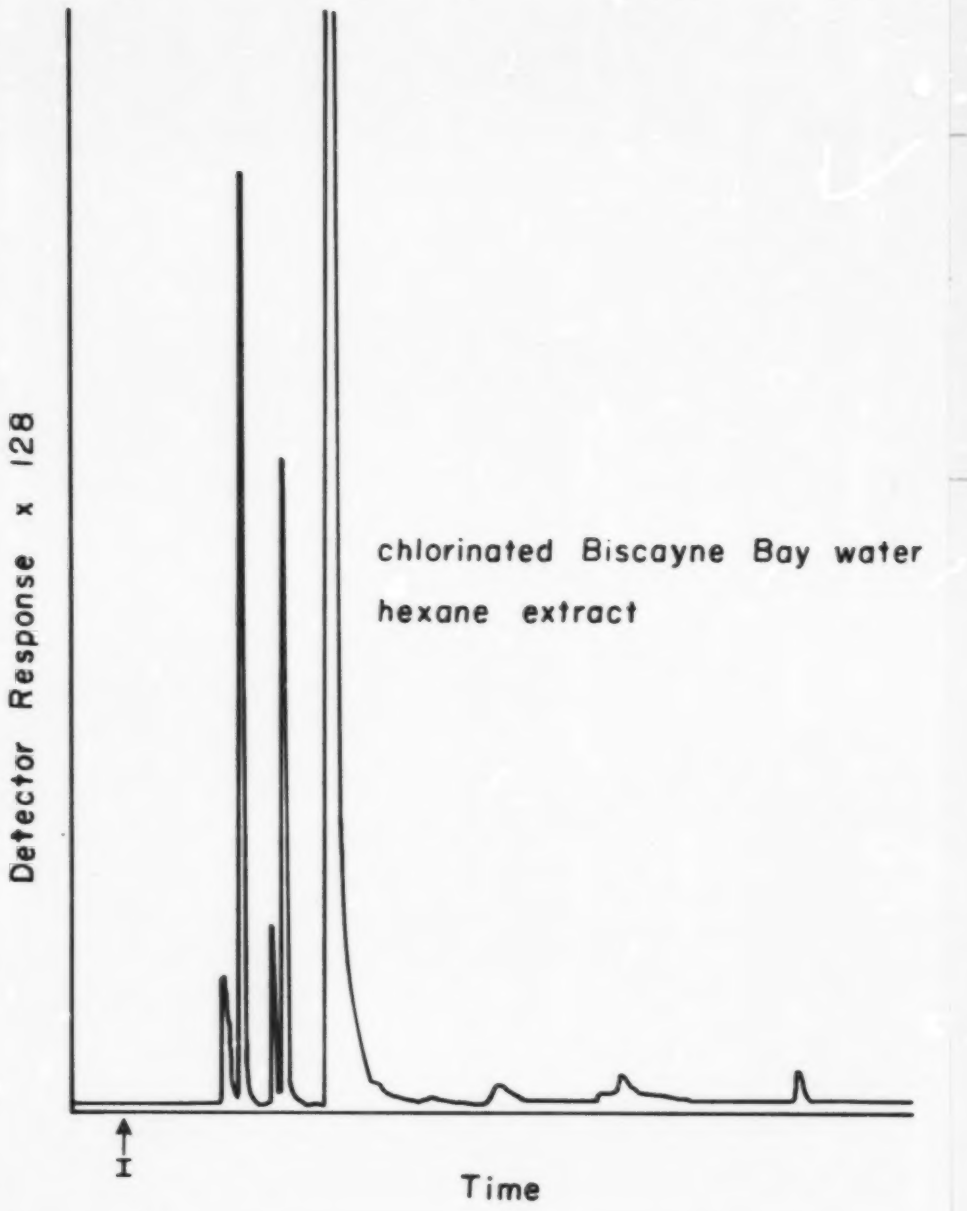

Figure 9c. GLC chromatogram of hexane extract of chlorinated Biscayne Bay water.

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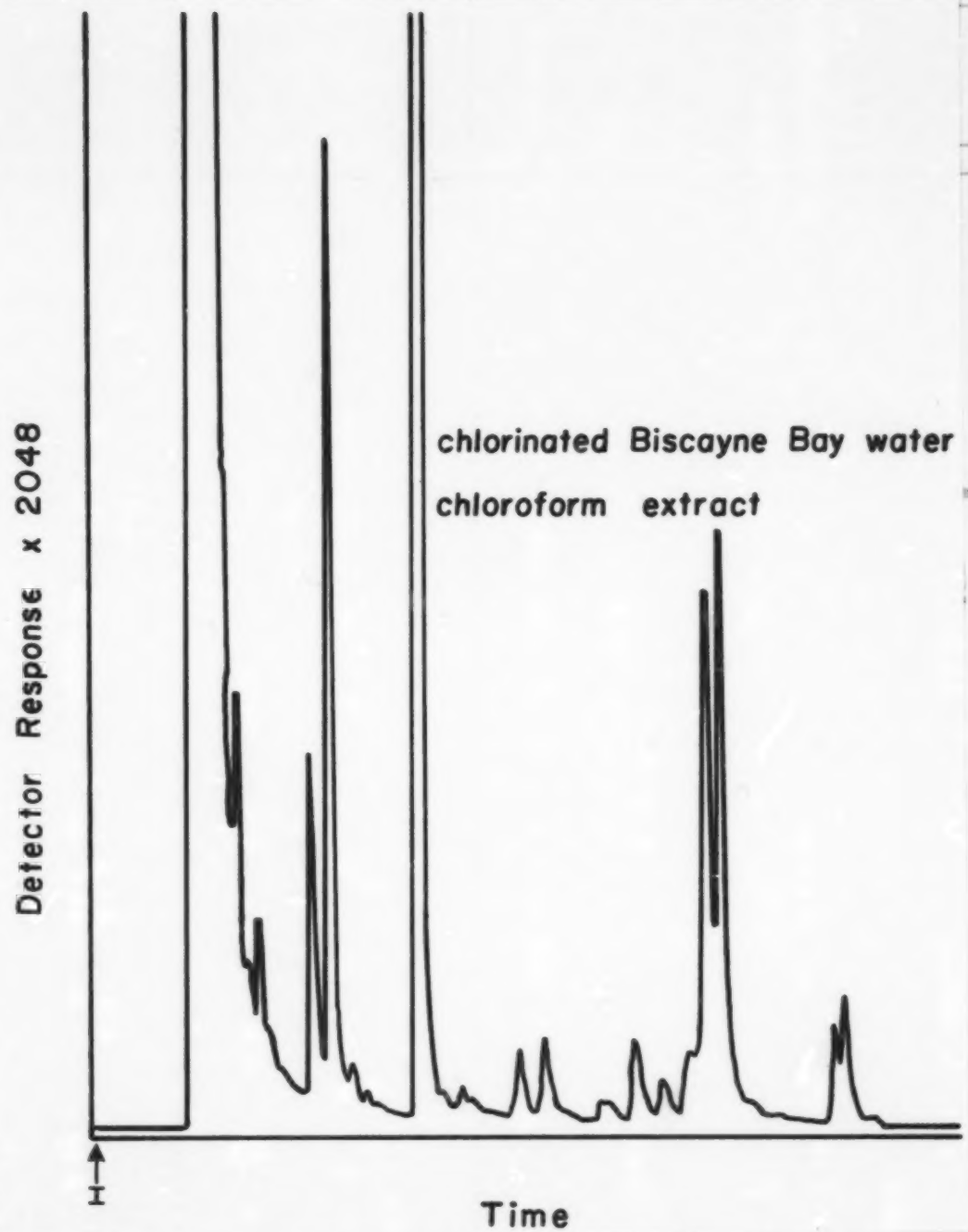

Figure 9d. GLC chromatograph of chloroform extract of chlorinated Biscayne Bay water.

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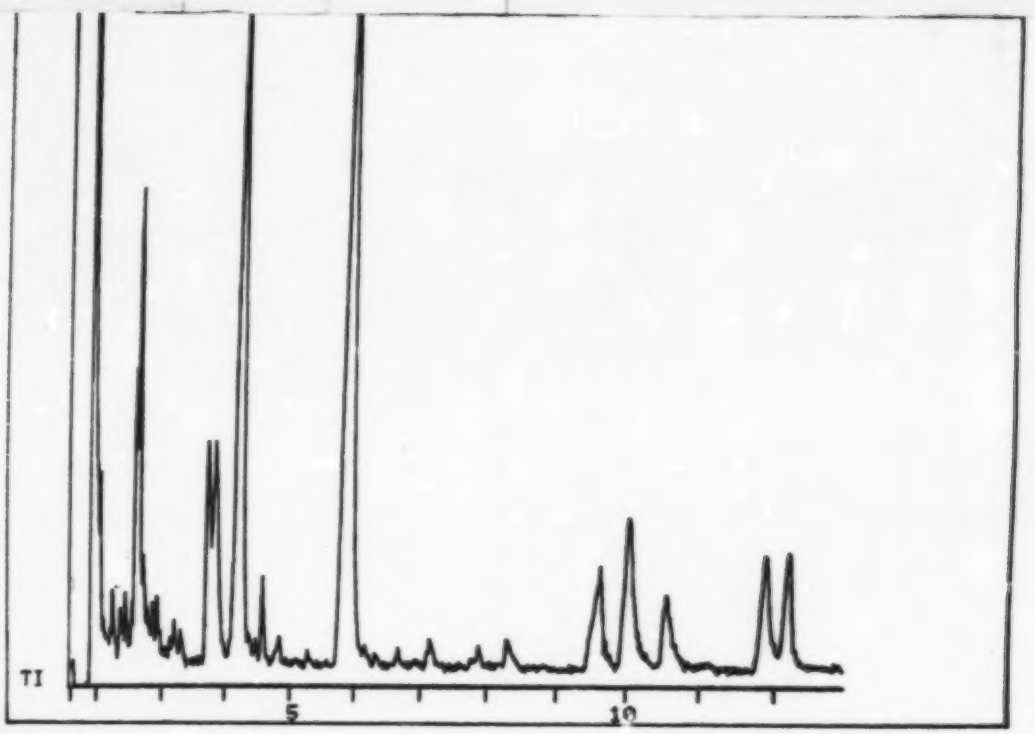

Figure 10. Total ion chromatogram of chloroform extract of chlorinated Biscayne Bay water, total ion abundance vs. elution time, minutes.

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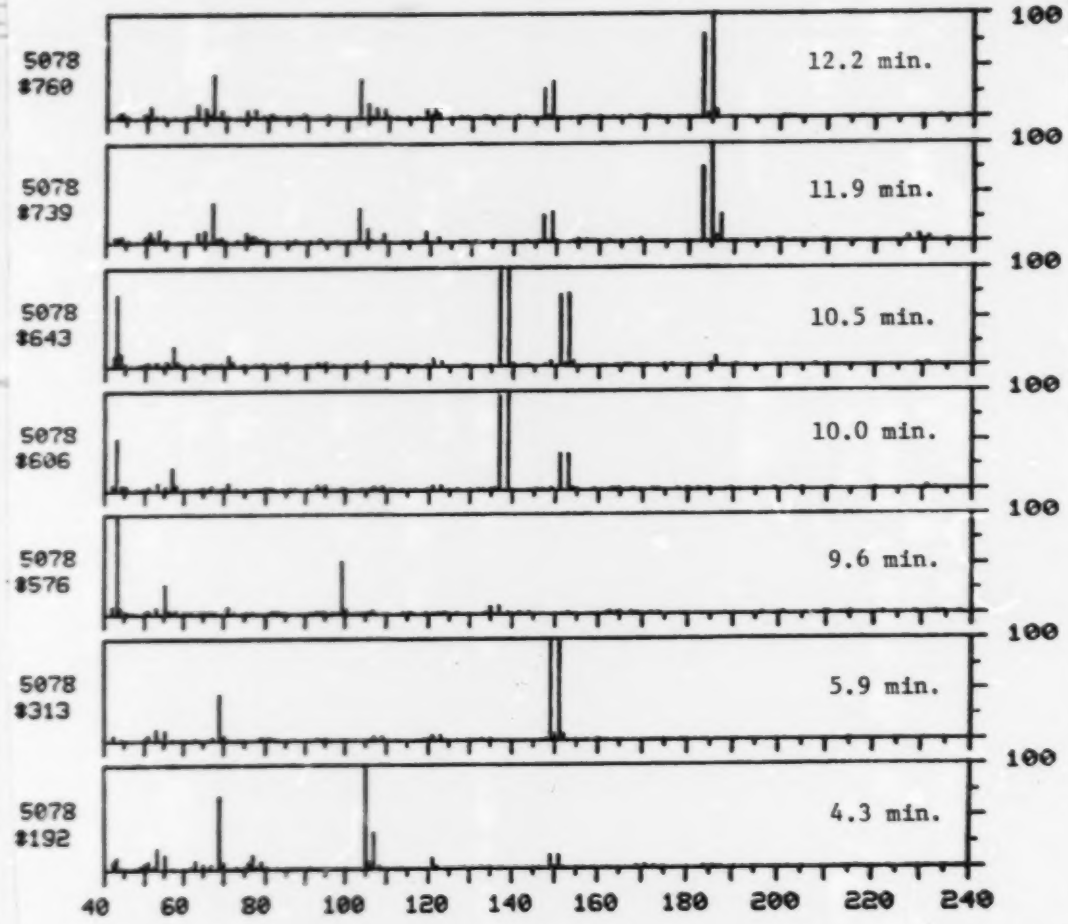

Figure 11. Mass spectra of major peaks shown in Figure 10, relative abundance vs. atomic mass units.

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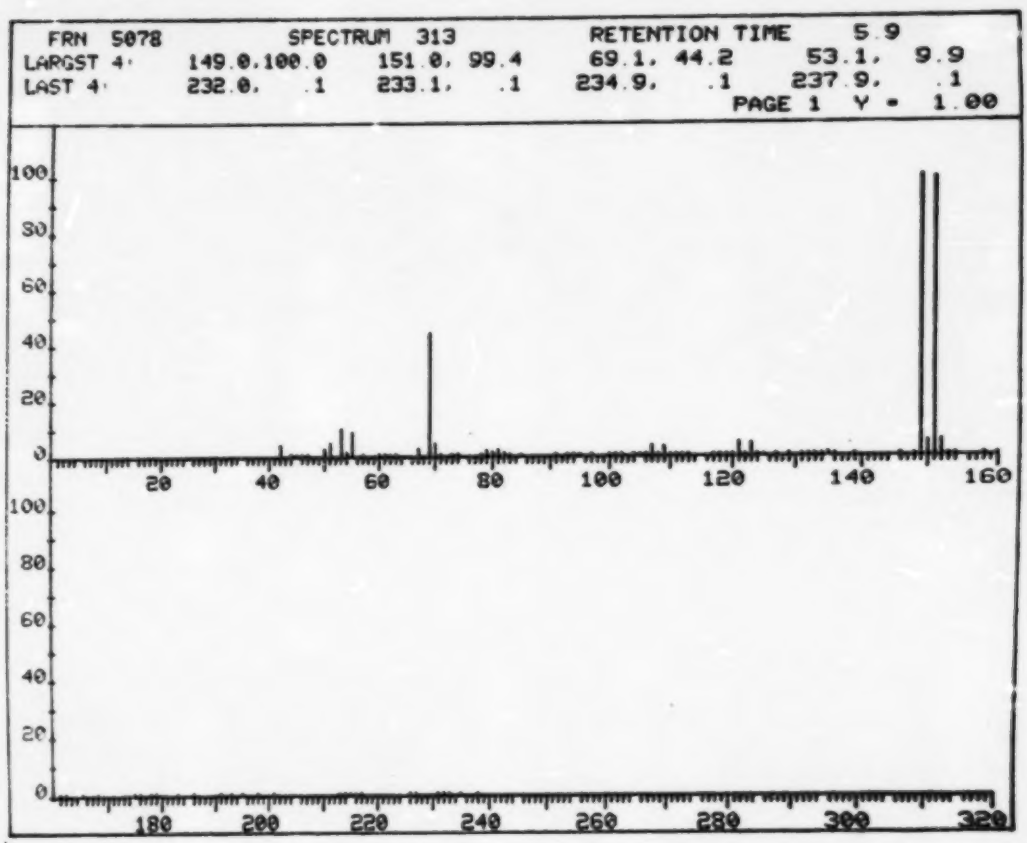

Figure 12. Mass spectrum of peak at 5.9 minutes, relative ion abundance vs. atomic mass units.

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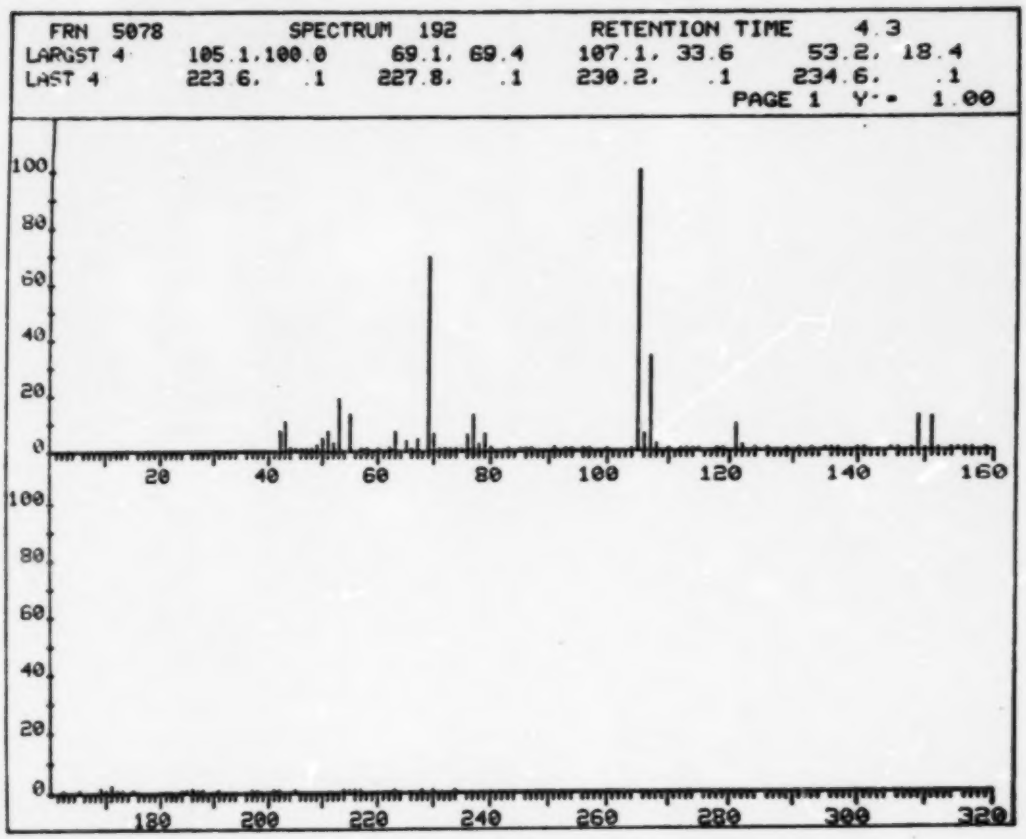

Figure 13. Mass spectrum of peak at 4.3 minutes, relative ion abundance vs. atomic mass units.

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Compounds represented by the two major peaks at 4.2 and 5.9 min were studied further due to their abundance. GC/MS runs at low energy (20 EV) electron impact mode (EI) and in the chemical ionization mode (CI) (methane reactant gas) did not reduce the m/e 149/151 couplet, nor were there any new ions observed at higher masses. Lacking any guidance from the literature to the contrary, the hypothesis was taken that m/e 149 represented a true molecular ion accepting that confirmation was not obtained by CI mass spectrometry (i.e., M + 1 and M + 15 ions were not observed with methane). Accepting this assumption, the 5.9 min peak of the 30 January 1978 chloroform extract can be represented by an elemental composition of C_4H_8NBr. The m/e 69 fragment suggested a neutral loss of HBr, a common neutral fragment in EI MS. It appears that the compound could be an isomer of bromopyrrolidine:

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Further, the 4.3 min peak of the 30 January 1980 was given a hypothetical structure of $C_5H_8NO_2Cl$, or chloroproline on the basis that the m/e 44 neutral (149-150 m/e) was COO and that the m/e isotope ratio of 100/30 for m/e 105/107 is chlorine.

This rationale suggested the investigation of the halogenation of naturally occurring amino acids, especially proline, as a direct step toward the synthesis and chemical characterization of the major compound found in the chloroform extract of chlorinated Biscayne Bay water.

8.c. PREPARATION AND IDENTIFICATION OF UNKNOWNS FOUND IN CHLOROFORM EXTRACTS

8.c.1. Synthesis of Unknown Compound

Early efforts to synthesize a compound having a mass spectrum matching that of the major compound found by GC/MS in the chloroform extract of the chlorinated 14 December 1977 Biscayne Bay water (i.e., m/e 69, 149, 151) were founded on the premise that m/e 149 was a true molecular ion. Interpretation based upon that premise led to the prediction that the compound was bromopyrrolidine (with the location of the bromine left unstated) and that the precursor of the compound was a simple five membered cyclic secondary amine. Proline and pyrrolidine were considered as possible candidates, but only the former has been observed in natural waters. An early experiment in which proline-spiked Gulf Stream water was chlorinated and extracted with chloroform produced a compound which matched the mass spectra of the unknown served to support that direction. The most direct approach to the synthesis of the compound seemed to be the bromination of pyrrolidine. This was done

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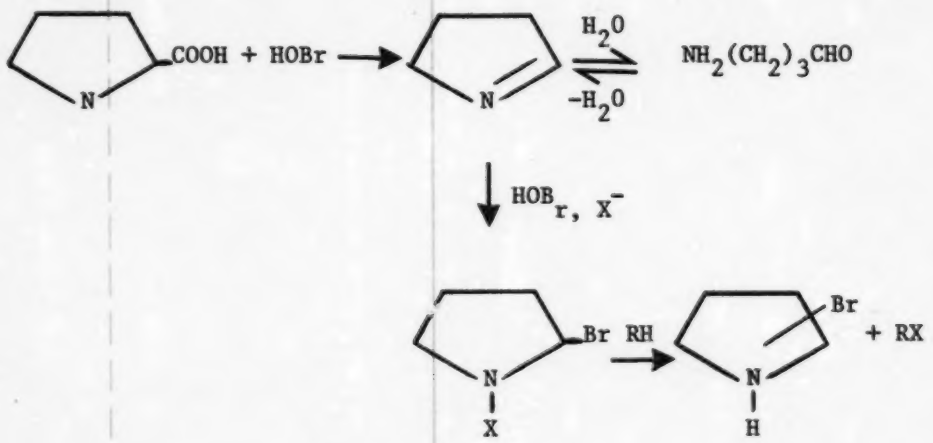
successfully by adding bromine in sodium hydroxide solution directly to pyrrolidine in 0.1M phosphate buffer with subsequent extraction using chloroform. This method was successful, but it provided very low yields. Subsequently, a variety of approaches were taken including the halogenation of pyrrolidine by light-induced free radicals from N-halosuccimide (Cl and Br), the Hunsdiecker rearrangement of the silver salt of proline, and the halogenation by N-halosuccimide in various solvent and temperature combinations. Halogenation of aqueous solutions of proline confirmed that decarboxylation occurs as a first or early step in the oxidation of that amino acid. In general, however, aqueous oxidation of proline by HOBr followed by extraction with chloroform did not prove to be a reliable synthesis method. Chloroform has a number of undesirable properties as an extraction solvent, i.e. probably toxicity, relatively high boiling point, possible reactivity to aqueous free radical attack, etc. As a result, an alternate solvent was sought. Hexane was not successful in baywater extracts (reported in section 8b); neither were saturated halogenated solvents such as carbon tetrachloride, and 1,1,2,trichlorotrifluoroethane in laboratory extracts of halogenated proline. Ethyl ether (ethanol stabilized or unstabilized) appeared to have sufficient advantages to make it the solvent of choice. These advantages included higher extraction efficiency, high volatility (lower distillation temperatures, very rapid sample preparation), and much improved chromatographic properties.

Concurrent postulation of reaction mechanisms suggested that an important intermediate in the formation of the haloamine from proline might be the cyclic imine, 1-pyrroline:



(X = Cl or Br)

Supporting evidence for the the imine's equilibrium product, 4-aminobutryaldehyde, is shown in section 8.c.4. Since increasing the equilibrium concentration of 1-pyrroline is favored by removing the water, it was considered advantageous to conduct the reaction in non-aqueous media. Also, it has been reported (34) that HOCl (and presumable HOBr) can be extracted into ether. Consequently, the following procedure was devised and was found to be consistently successful:

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Step 1: Dissolve proline to 0.005 molar in 200 ml of buffered "matrix" (0.3M Cl^-, 0.3M Br^-, 0.1M borate buffer to 8.1 pH).
Add sufficient NaOCl solution to ca 1.5X molar excess.

Step 2: Dissolve sufficient NaOCl in 200 ml of the "matrix" to give ca 5-10X molar excess to proline. Adjust pH to 8.1.

Step 3: Extract solutions from Steps 1 and 2 separately with HPLC grade ethyl ether, then add extract from Step 2 dropwise to extract from Step 1.

Step 4: Dry the combined ether extract over anhydrous sodium sulfate and concentrate to 1 ml using Kuderna-Danish and micro-Snyder glassware.

Step 5: Isolate and purify the compound using gas chromatography (GLC) with a 3 mm ID, 2 meter OV-101 packed column with a temperature program of 55°C isothermal for 4 minutes and then increasing at 16°C per minute to 90°C hold, and a helium carrier gas flow rate of 60 ml/min. Inject 30 microliters of the concentrated solution from step 4. Monitor the discharge with a thermal conductivity detector and manually trap the fraction containing the peak at 7.4 minutes in a dry-ice cooled U-tube microtrap. Transfer the collected liquid material to a cone-shaped reaction vial with a septum cap.
Rechromatograph the collected material isothermally at 80°C and collect by trapping the material from the peak at 5.0 minutes. Use 4 microliter aliquots of the material that was collected in the reaction vial. Transfer the material to a 1 ml sealable glass ampule and dry over P_2O_5 to constant weight before sealing. Commonly, yields of 5 milligrams were found for this overall and rather tedious procedure.

8.c.2. Elemental Analysis of Synthesized Unknown Compound

Elemental analyses of the synthesized unknown compound were performed by Galbraith Laboratories, Inc. on two samples of the compound that matched both the retention time and mass spectrum of the major unknown in chloroformanalytical schemes, normally the minimum sample is roughly 30 to 50 milligrams. Several months of effort were devoted to preparing the required amount of material through the use of the above outlined preparative scale gas chromatography. The results are shown in Table 5. No chlorine was found as might be expected since bromide ion oxidation in chlorinated seawater is rapid as described above. The absence of nitrogen indicates that the cyclic amine structure in the proline is not present in the reaction product. Possible interpretation of these results is discussed in conjunction with additional mass spectral data below.

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TABLE 5. ELEMENTAL ANALYSIS OF BROMINATED UNKNOWN

Sample	Elemental Analysis						
#	%C	%H	%N	%Br	%Cl	%O	
M2-146	22.83	4.02	*	*	*	*	-
M3-12	19.90	3.97	0.58	64.16	0.5	*	11.4

* Not determined due to insufficient sample.

Performed by Galbraith Laboratories, Inc., 2323 Sycamore Drive, Knoxville, TN 37921.

8.c.3. NMR Spectra

NMR spectra of the synthesized unknown suggest a compound having three methyl groups, i.e., nine protons, one methyl being split by a methine group at low field. A typical 60MHz ^{1}H NMR spectrum is shown in Figure 14. The peaks at 3.7-3.9 appear to be composed of two singlets and a doublet. The doublet is not obvious until the quartet at that value is decoupled (not shown) causing a disappearance of the doublet and the appearance of two singlets of about equal magnitude. The interpretation of the spectra is not straightforward because an isomeric mixture may be present. This possibility is indicated because repetition of synthesis and chromatography produced materials that had somewhat different NMR spectra. The probable presence of isomers was indicated also in the GC/MS analyses in that several of the small peaks in the chromatograms gave the same major ions as did the primary large peak.

8.c.4. Fluorescence and Electrochemical Analyses of Chlorinated Proline

The chlorination of proline in pH 8.1 borate buffered chloride/bromide matrix solution was studied using two different polarographic procedures systems in the differential pulse mode (DDP), and two fluorescence procedures one with dansyl chloride, the other fluorescamine (Fluram). As shown in Figure 15, proline concentration was essentially reduced to zero at a molar ratio of 3.0 (Cl/proline) as measured by dansyl chloride and at this same molar ratio, an unkown product appeared to be at its maximum. Fluorescamine (Figure 15B) which reacts with primary amines only, indicated a maximum yield at a molar ratio of 1.5-2.0 (Cl/proline) of an unknown product which is probably the same as observed with the dansyl chloride. Theory suggests that this is (as shown in 8.c.2.) 4-amino-butryaldehyde. Differential pulse polarography shown in Figure 15 C and D show reduction waves for three unknown species with maxima at molar ratios of 1-2 (-1.1 to -1.2 volts SCE) and 3 (-1.5 volts SCE). An easily reducible species (-0.2 to -0.3 volts SCE) appears at a molar ratio of ca 2 with no maximum observed. Interpretation of these results

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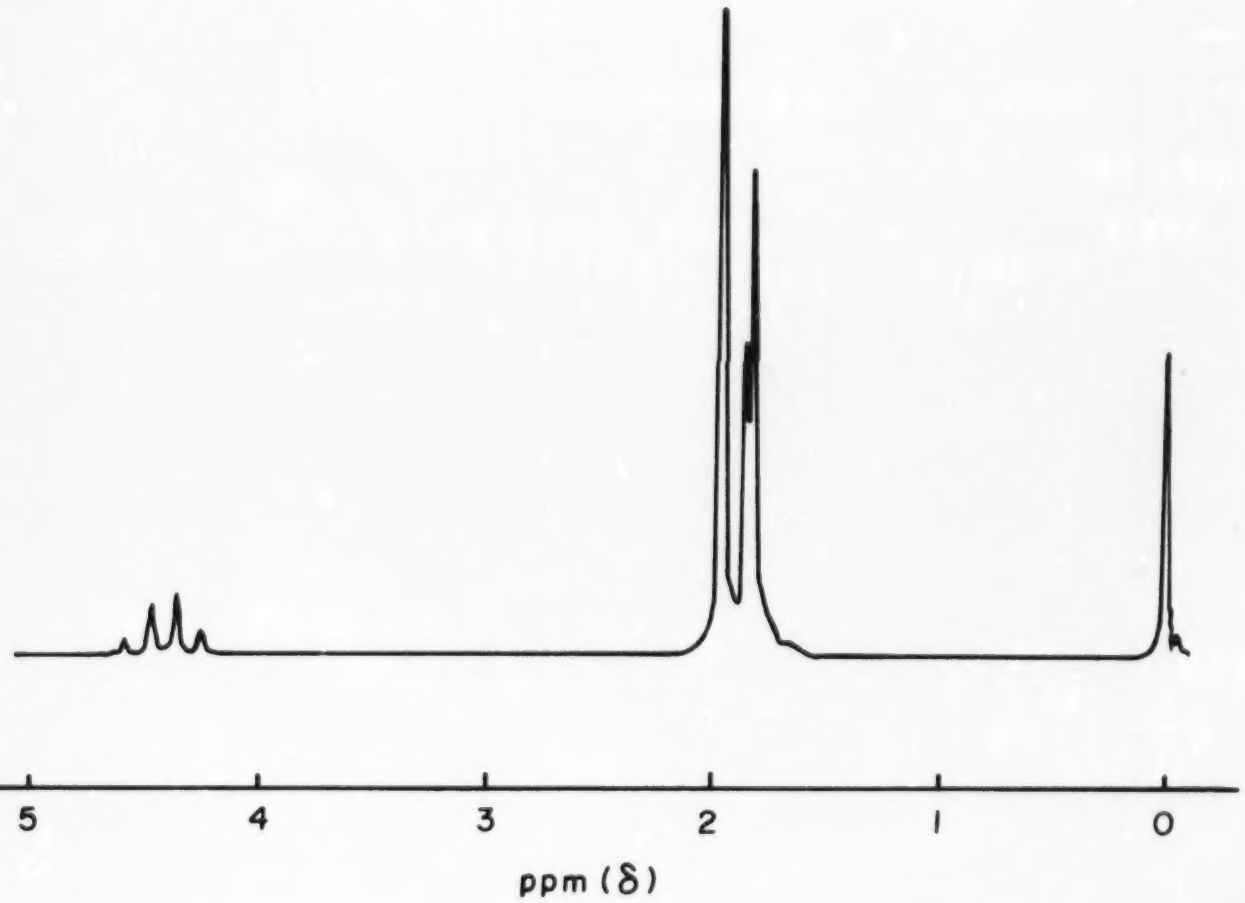

Figure 14. Proton NMR spectrum of unknown compound having m/e 69,149,151 mass spectrum (60mhz, ^{1}H, $CDCl_3$, Varian 360A, single scan, TMS internal reference).

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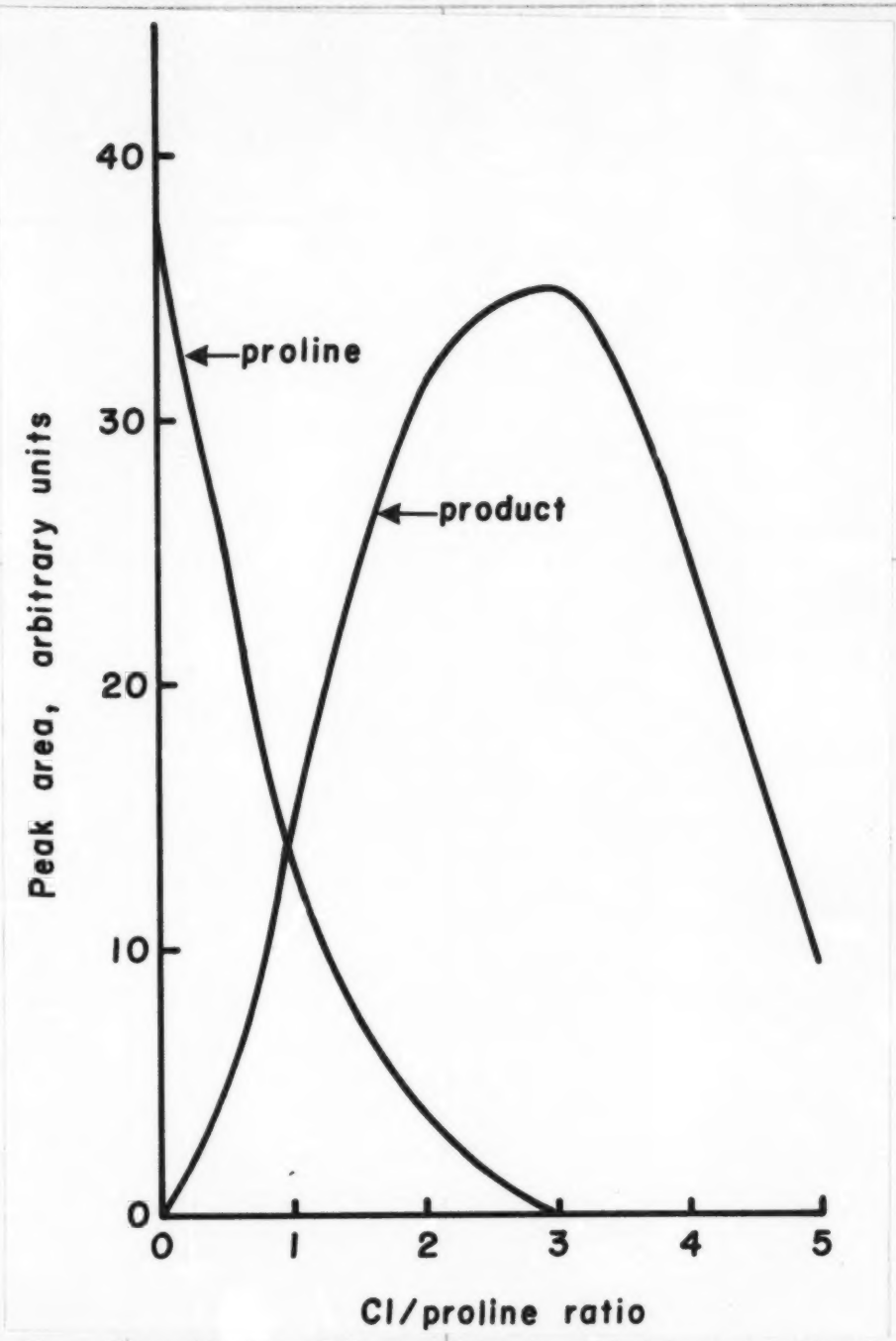

Figure 15a. The oxidation of 5 mM proline in pH 8.1 borate buffered chloride/bromide solution - dansyl chloride derivative separated by HPLC.

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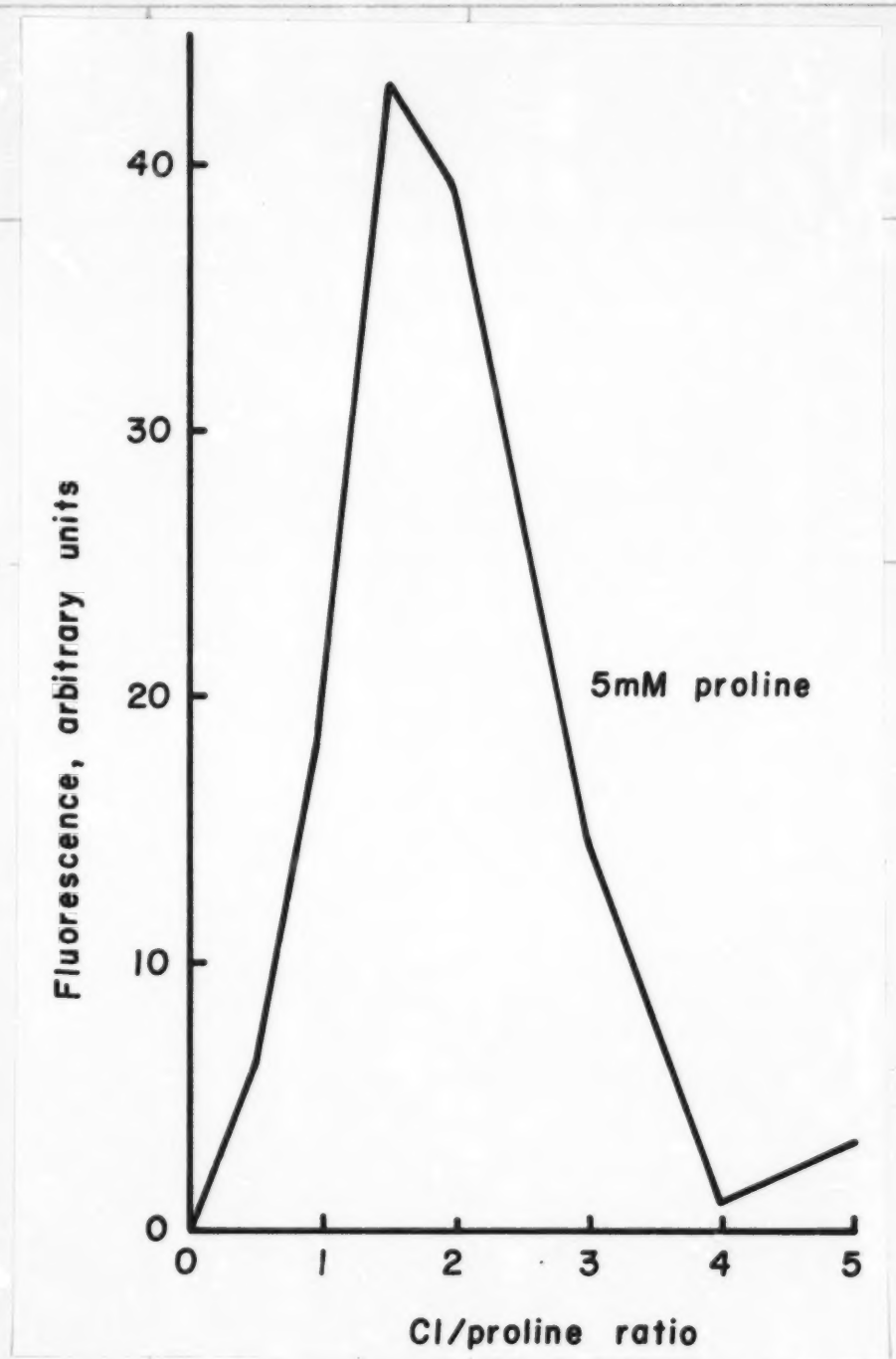

Figure 15b. The oxidation of 5 mM proline in pH 8.1 borate buffered chloride/bromide solution - measured by fluorescence of fluorescamine (Fluram) derivatives.

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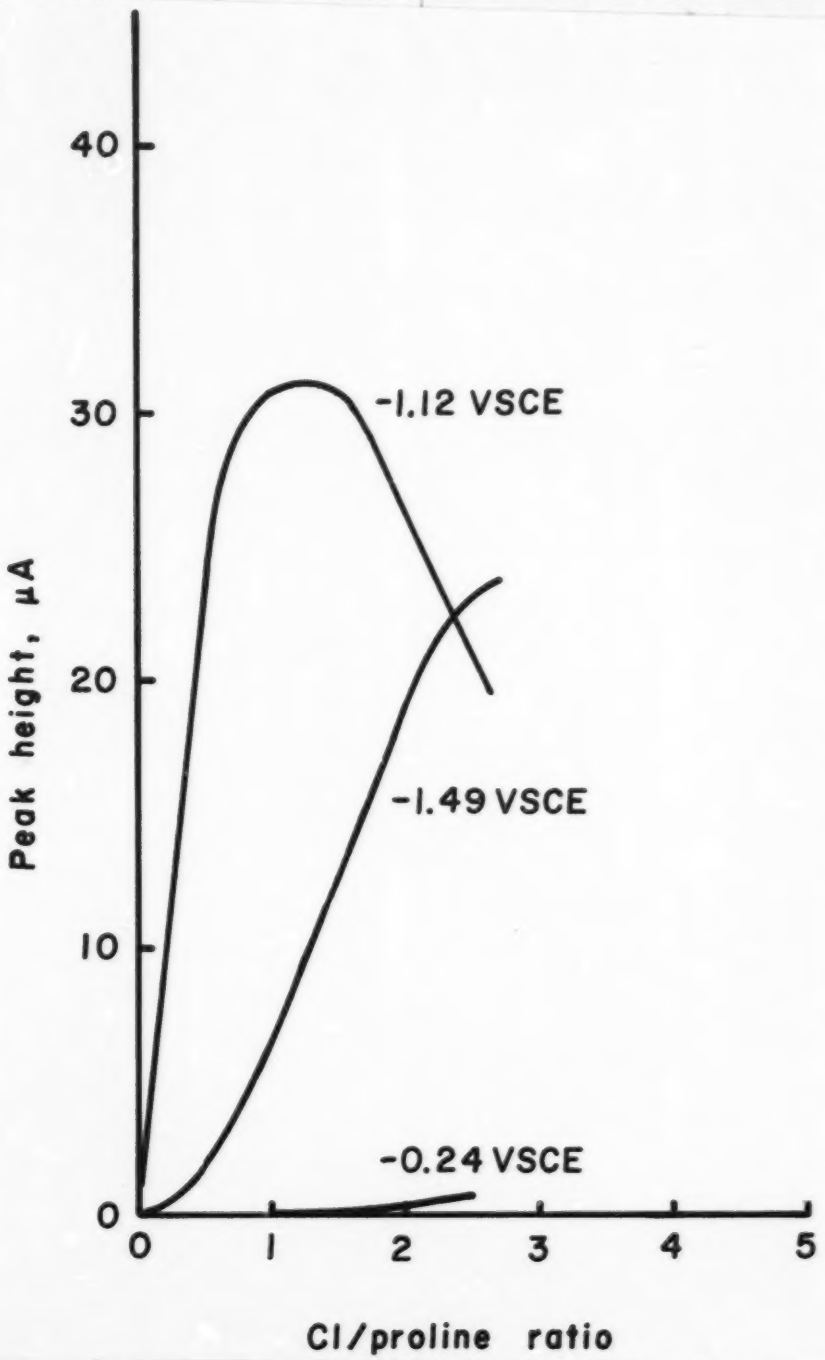

Figure 15c. The oxidation of 5 mM proline in pH 8.1 borate buffered chloride/bromide solution - measured by differential pulse polarography, PAR model 384.

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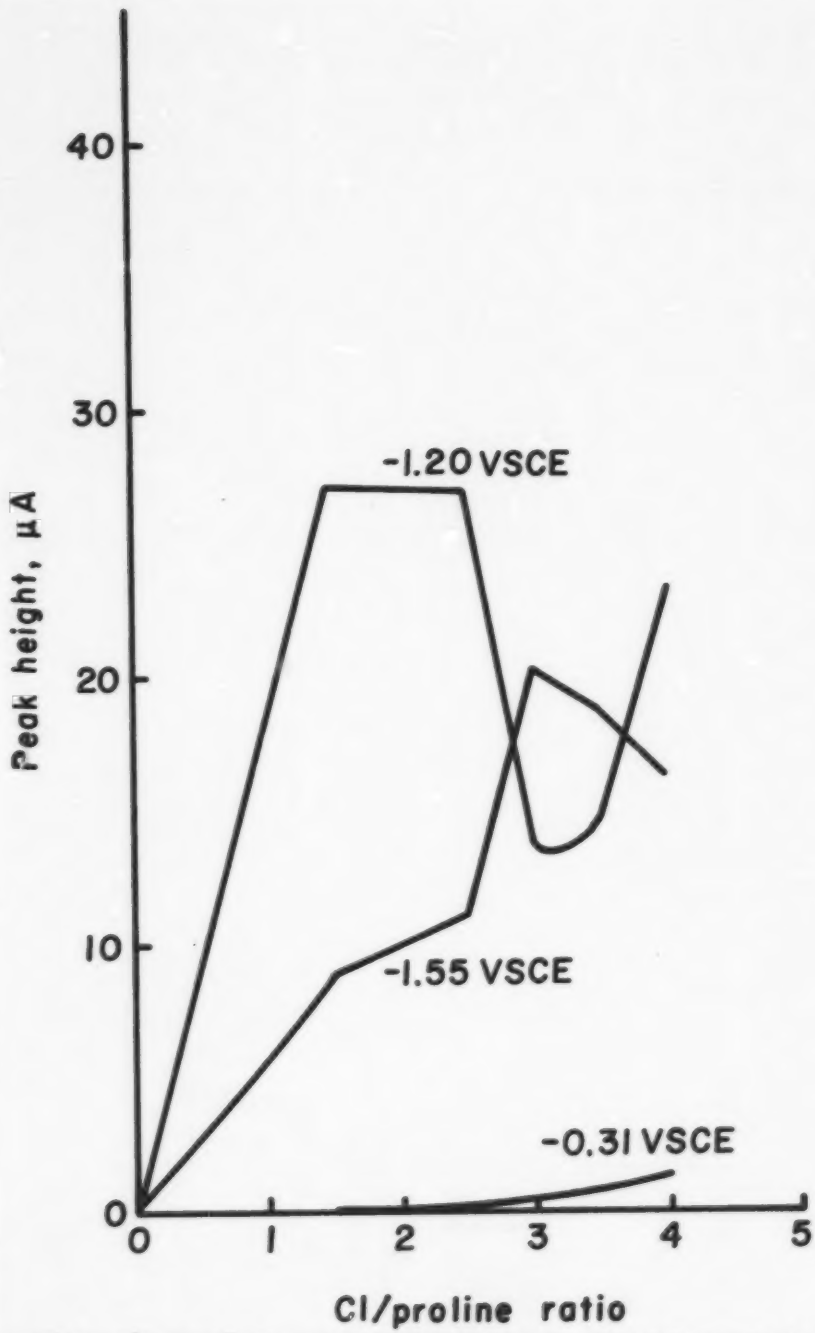

Figure 15d. The oxidation of 5 mM proline in pH 8.1 borate buffered chloride/bromide solution - measured by differential pulse polarography, PAR model 174.

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is presently limited to the recognition that proline is oxidized by increased amounts of halogen and that new chemical species appear and in some cases disappear with increased halogen addition. The use of DPP and fluorescamine and dansyl chloride derivatization shows promise for future experimental schemes.

8.c.5. Possible Artifacts

Chloroform, or some contaminant or stabilizer (usually ethanol), was considered as a possible source of the unknown compounds observed. Chloroform is known to participate in free radical reactions, and the ingredients for such reactions (heat, light, aqueous interphase) were present. The probability of artifact compounds from chloroform appears low since chloroform extracts of Gulf Stream water did not produce the unknown compounds. Further, chloroform treated directly with aqueous HOBr also produced negative results. Co-extraction of HOBr and the organic precursor(s) with subsequent reaction in the non-aqueous phase cannot be totally ruled out, but no evidence for such reactions has been found. Alternate analytical schemes that eliminate the extraction step such as differential pulse polarography (DPP) are attractive in this regard and could provide an approach to the study of these "as yet to be identified" chlorination products.

8.c.6. Limited Search for Molecular Ions

While the chemical ionization results and the stability of the m/e 149-151 couplet as the ionization potential was varied suggested that the m/e 149-151 couplet was possibly a molecular ion, the failure to find nitrogen in the elemental analyses denies the idea that this odd-numbered couplet was a molecular ion. Based on the results of the elemental analyses, alternate empirical formulas that had oxygen in place of the previously hypothesized nitrogen and the presence of two bromine atoms were considered. The GC/MS system was operated in the more sensitive selection ion monitoring (SIM) mode and a peak at m/e 228 and smaller peaks at 230 and 232 were observed. Further work on elucidating the structure of the compound should be based on a more effective synthesis scheme to provide larger quantities of the material, which should improve the accuracy of the elemental analysis and NMR spectra and allow a conventional molecular weight determination. In particular, lanthanide shift analysis with the NMR would be extremely valuable.

8.d. ELECTROCHEMICAL ANALYSES OF CHLORINATED BAYWATER

An exploratory study was made of the use of differential pulse polarography (DPP) as an alternate method of detecting reaction products of the chlorination of seawater, by passing the solvent extraction step. In a very limited series of experiments, a DPP reduction wave was obtained at ca -0.9 v (SCE) upon chlorination that was reduced with time, as shown in Figure 16. The observed response did not appear in the blank (unchlorinated) nor does it correspond to analyses on chlorinated organic-free seawater or proline-spiked seawater. DPP is sufficiently sensitive to detect reducible species in situ

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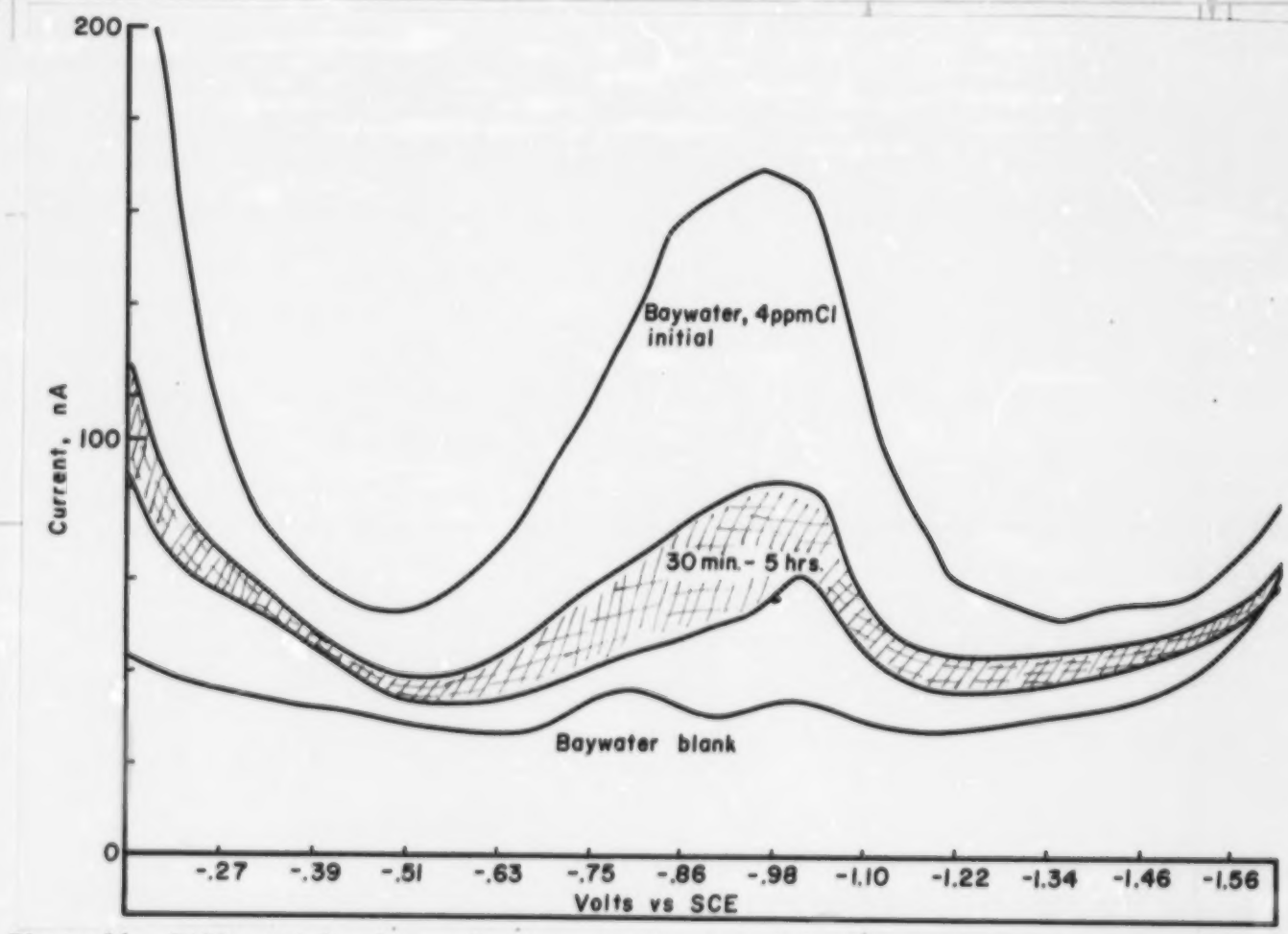

Figure 16. Differential pulse polarogram of chlorinated (1 ppm added) Biscayne Bay seawater showing blank (unchlorinated), immediately after chlorination, and after 30 minutes to 5 hours.

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that are generated by chlorination, and are probably organic. The promise that DPP offers is that of a rapid, low cost assay for reducible species resulting from chlorination. Much work remains, however, before the reducible species can be identified.

8.e. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ON CHLORINATED AMINO ACIDS

Chlorination of amino acids in seawater cause a reduction in the concentration of the amino acid and in some cases produces reaction products that form dansyl derivatives, based upon a brief study using high performance liquid chromatography (HPLC) with fluorescence detection. As shown in Table 6 of the eleven amino acids studied, all were reduced in concentration to some degree, from 29 percent for hydroxy-proline to 90 percent for lysine after reaction with a 3X molar excess of HOCl in pH 8.1 borate buffered Gulf Stream sea water. Of the amino acids studied, three produced unambiguous new peaks in the fluorescence chromatograms. Separations were made on reverse phase RP-18 columns with methanol-water gradient elution. Amino acids are attacked by free halogen in seawater and in some cases form unknown derivatives that may be of interest. Variables such as time, concentration, light, temperature, and pH remain to be studied.

8.f. SUMMATION

Our work shows that GC/MS analysis of chloroform extracts of chlorinated Biscayne Bay water reveals new halogenated compounds that cannot be dismissed as artifacts, nor have they been amenable to laboratory synthesis on a scale sufficient to obtain a satisfactory chemical description. It is reasonable to conclude that these compounds are "residual oxidants" with finite lifetimes in natural waters with unknown toxicities and environmental impacts. The precursors of these compounds, and their ubiquity in natural waters, remains to be defined. Each analytical method provides clues to the chemical processes that chlorination incurs, but a clear picture has not yet emerged. The elucidation of the exact chemical structures of "residual oxidants" presents a tantalizing challenge that could be developed using the discoveries described above as a foundation. Another approach in which fractions of natural extracts are characterized according to their halogen content, redox properties, and biological response may be productive also from an environmental viewpoint.

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TABLE 6. CHLORINATED AND UNCHLORINATED AMINO ACIDS - HPLC SEPARATIONS OF DANSYL DERIVATIVES

Amino Acid	Percent Reduction with Chlorination	Number of New Compounds
hydroxy-proline	29	2
glycine	43	0
alanine	35	0
phenylalanine	58	0
tryosine	78	0
lysine	90	3
methionine	92	0
cysteine	33	0
tryptophan	60	1
leucine	66	0
isoleucine	76	0

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